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# Sweat Testing: Sample Collection and Quantitative Chloride Analysis; Approved Guideline—Third Edition

This document addresses appropriate methods of collection and analysis, quality control, and the evaluation and reporting of test results.

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A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.



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*Advancing Quality in Health Care Testing*

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### Abstract

Clinical and Laboratory Standards Institute document C34-A3—*Sweat Testing: Sample Collection and Quantitative Chloride Analysis; Approved Guideline—Third Edition* is a guideline for the performance of the sweat test for the diagnosis of cystic fibrosis. The primary audience includes laboratory and clinical personnel responsible for collecting, analyzing, reporting, and evaluating sweat test results. Sweat stimulation, collection, and the quantitative measurement of sweat chloride are described along with quality assurance and result evaluation.

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## Foreword

The quantitative measurement of chloride in sweat (commonly called the “sweat test”) is used to confirm the diagnosis of cystic fibrosis (CF). With an approximate incidence of 1:3200, CF is the most common life-threatening genetic disease within the white population. It is an autosomal recessive disorder characterized by viscous secretions that affect the exocrine glands, primarily in the lungs and pancreas. Patients with CF have an increased concentration of sodium, chloride, and potassium in their sweat. The criteria for the diagnosis of CF include the presence of one or more characteristic phenotypic features, or a history of CF in a sibling, or a positive newborn screening test result; and an increased sweat chloride concentration by pilocarpine iontophoresis on two or more occasions, or identification of two CF-causing mutations or demonstration of abnormal nasal epithelial ion transport.<sup>1,2</sup>

The sweat test has been reported to have unacceptably high false-positive (up to 15%) and false-negative (up to 12%) rates attributable to inaccurate methodology, technical error, and patient physiology.<sup>3-8</sup> Comprehensive guidelines addressing the collection of sweat and the quantitative measurement of chloride in sweat are needed. Improvement in the performance of such tests can only occur when laboratory scientists and clinicians are aware of appropriate methods of collection and analysis, quality control, and evaluation of results. This document describes, in detail, the quantitative pilocarpine iontophoresis test for the determination of sweat chloride, including techniques to minimize the potential for false-positive and false-negative test results. Screening methods based on sweat conductivity are also mentioned. Other methods for measuring sweat electrolytes after pilocarpine iontophoresis exist but are not included in the guideline. Some of these methods are documented as having significant analytical problems.<sup>3-8</sup>

The Cystic Fibrosis Foundation requires that, at accredited Cystic Fibrosis Care Centers for diagnosis, sweating be stimulated by pilocarpine iontophoresis and collected in either gauze or filter paper, or microbore tubing followed by quantitative measurement of chloride.<sup>2</sup> At alternative sites, as a screening procedure, conductivity may be measured (see Section 10). Patients with a sweat conductivity value of 50 mmol/L (equivalent NaCl) or above should have a quantitative measurement of sweat chloride.<sup>9,10</sup>

This edition replaces the second edition approved guideline, C34-A2, which was published in 2000. Several changes have been made in this edition, including the following additions: a microvolume chloride procedure for sweat collected in coils; storage conditions for sweat; new reference ranges for infants; suggestions for enhancing sweat collection volume. It also includes sections on method validation and on developing and monitoring quality assurance and quality control.

## Key Words

Chloridometer, iontophoresis, sweat chloride, sweat testing



# Sweat Testing: Sample Collection and Quantitative Chloride Analysis; Approved Guideline—Third Edition

## 1 Scope

The following procedures are described: the stimulation and collection of sweat and the quantitative measurement of chloride; sweat stimulation by pilocarpine iontophoresis (specific precautions are noted); and sweat collection in filter paper, gauze, and microbore tubing. Sweat chloride ( $\text{Cl}^-$ ) determination is described using coulometric titration. Screening methods based on sweat conductivity are also mentioned. Other methods for measuring sweat electrolytes after pilocarpine iontophoresis exist but are not included in the guideline. Some of these methods are documented as having significant analytical problems and also have limited diagnostic application.<sup>3-8</sup> Validation studies and quality assurance (QA) techniques are discussed, along with analytical and biological sources of error. The evaluation of sweat chloride test results to include reference intervals and diagnostic criteria are described, with an emphasis on the application of sweat chloride testing to newborn screening for cystic fibrosis (CF). This document is primarily directed towards laboratory and clinical personnel responsible for collecting, analyzing, reporting, and evaluating sweat chloride test results.

Because the sweat test has been reported to have unacceptably high false-positive and false-negative rates attributable to inaccurate methodology, technical error, and patient physiology,<sup>3-8</sup> comprehensive guidelines addressing the collection of sweat and the quantitative measurement of chloride in sweat are needed. Improvement in the performance of such tests can only occur when laboratory scientists and clinicians are aware of appropriate methods of collection and analysis, quality control (QC), and evaluation of results. This document describes, in detail, the quantitative pilocarpine iontophoresis test for the determination of sweat chloride, including techniques to minimize the potential for false-positive and false-negative test results.

## 2 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to “standard precautions.” Standard precautions are guidelines that combine the major features of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of all infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the US Centers for Disease Control and Prevention.<sup>11</sup> For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to CLSI document M29.<sup>12</sup>

Currently, standard precautions for protection from transmissible infectious agents exempt sweat unless it contains visible blood. However, it is recommended that laboratory personnel wear powder-free gloves during sweat collection and analysis as routine practice, both for their protection and to prevent contamination of the sample.<sup>11</sup>

## 3 Terminology

### 3.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization whenever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the

global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in CLSI, ISO, and CEN documents; and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. In light of this, CLSI's consensus process for development and revision of standards focuses on harmonization of terms to facilitate the global application of standards and guidelines.

In order to align the usage of terminology in this document with that of ISO, the term *accuracy*, in its metrological sense, refers to the closeness of the agreement between a measured quantity value and a true quantity value of a measurand, and comprises both random and systematic effects. *Trueness* is used in this document when referring to the closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value; the measurement of trueness is usually expressed in terms of *bias*. *Precision* is defined as the "closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions." As such, it cannot have a numerical value, but may be determined qualitatively as high, medium, or low. For its numerical expression, the term *imprecision* is used, which is the "dispersion of results of measurements obtained under specified conditions." In addition, different components of precision are defined in C34-A3, primarily *repeatability*, ie, "precision under conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time" while *reproducibility* describes "precision under conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment." For the sake of introduction and to avoid confusion, the subcommittee chose to include the ISO terms parenthetically where the US terms appear.

The term *measurand* (quantity intended to be measured [ISO/IEC Guide 99]), is used in combination with the term *analyte* (component represented in the name of a measurable quantity) when its use relates to a biological fluid/matrix; the term *analytical measuring interval* in combination with *analytical measurement range* when referring to a set of values of quantities of the same kind that can be measured by a given measuring instrument or measuring system with specified instrumental uncertainty, under defined conditions (ISO/IEC Guide 99); and the term *measurement procedure* has replaced *analytical method* for a detailed description of a measurement according to one or more principles and to a given method, based on a model and including any calculation to obtain a result.

Users of C34-A3 should understand, however, that the fundamental meanings of the terms are identical in many cases, and to facilitate understanding, terms are defined in the Definitions section of this guideline.

All terms and definitions will be reviewed again for consistency with international use, and revised appropriately during the next scheduled revision of this document.

### 3.2 Definitions

**accuracy (measurement)** – closeness of agreement between a measured quantity value and a true quantity value of a measurand (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 1:** The concept 'measurement accuracy' is not a quantity and is not given a numerical quantity value. A measurement is said to be more accurate when it offers a smaller measurement error (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 2:** The term "measurement accuracy" should not be used for measurement trueness and the term "measurement precision" should not be used for 'measurement accuracy,' which, however, is related to both these concepts (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 3:** 'Measurement accuracy' is sometimes understood as closeness of agreement between measured quantity values that are being attributed to the measurand (ISO/IEC Guide 99).<sup>13</sup>

**analyte** – component represented in the name of a measurable quantity (ISO 17511)<sup>14</sup>; **NOTE 1:** In the type of quantity "mass of protein in 24-hour urine," "protein" is the analyte. In "amount of substance of glucose in plasma," "glucose" is the analyte. In both cases, the long phrase represents the

measurand (ISO 17511)<sup>14</sup>; **NOTE 2:** In the type of quantity “catalytic concentration of lactate dehydrogenase isoenzyme 1 in plasma,” “lactate dehydrogenase isoenzyme 1” is the analyte. The long first phrase designates the measurand (ISO 18153)<sup>15</sup>; **NOTE 3:** The analyte is the particular component of interest to the patient; **NOTE 4:** This includes any element, ion, compound, substance, factor, infectious agent, cell, organelle, activity (enzymatic, hormonal, or immunological), or property, activity, intensity, or other characteristics of which are to be determined; **NOTE 5:** As used in this document, the pure molecular or cellular form of the substance to be detected or quantified, independent of the sample matrix in which it is present; **NOTE 6:** This is the chemical entity/substance that is actually intended to be measured; **NOTE 7:** Formerly in this document, analyte was used to describe both a single component (analyte) as well as the analyte in its specific matrix (measurand).

**analytical measuring interval (AMI)** – a set of values of quantities of the same kind that can be measured by a given measuring instrument or measuring system with specified instrumental uncertainty, under defined conditions (ISO IEC Guide 99)<sup>13</sup>; **NOTE:** Sometimes called the analytical measurement range (AMR), which is the range of analyte values that a method can directly measure on the specimen without any dilution, concentration, or other pretreatment not part of the usual assay process.<sup>16</sup>

**bias** – the difference between the expectation of the test results and an accepted reference value (ISO 3534-1).<sup>17</sup>

**calibrator** – measurement standard used in calibration (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 1:** For this document, a material or device of known, or assigned quantitative characteristics (eg, concentration, activity, intensity, reactivity, responsiveness) used to adjust the output of a measurement procedure or to compare the response obtained with the response of a test specimen and/or sample; **NOTE 2:** The quantities of the analytes of interest in the calibration material are known within limits ascertained during its preparation and may be used to establish the relationship of a measurement procedure’s response to the characteristic measured for all methods or restricted to some; **NOTE 3:** Calibration materials with different amounts of analytes may be used to establish a calibration or response “curve” over a range of interest.

**chloridometer** – a coulometric titrator used to measure chloride ion concentration.

**control (control material)** – a device, solution, or lyophilized preparation intended for use in the quality control process; **NOTE 1:** The expected reaction or concentration of analytes of interest are known within limits ascertained during preparation and confirmed in use; **NOTE 2:** Control materials are generally not used for calibration in the same process in which they are used as controls.

**imprecision** – dispersion of independent results of measurements obtained under specified conditions; **NOTE:** It is expressed numerically as **standard deviation** or **coefficient of variation**.

**iontophoresis** – the migration of small ions in an electrical field; **NOTE:** In the sweat test, pilocarpine is iontophoresed into the skin to stimulate sweating.

**limit of quantitation (LoQ)** – lowest amount of analyte in a sample that can be quantitatively determined with stated acceptable precision and trueness, under stated experimental conditions (modified from WHO-BS/95.1793).<sup>18</sup>

**linearity** – the ability (within a given range) to provide results that are directly proportional to the concentration {amount} of the analyte in the test sample (WHO-BS/95.1793)<sup>18</sup>; **NOTE 1:** Linearity typically refers to overall system response (ie, the final analytical answer rather than the raw instrument output; **NOTE 2:** The linearity of a system is measured by testing levels of an analyte that are known by formulation or known *relative to each other* (not necessarily known absolutely); when the system results are plotted against these values, the degree to which the plotted curve conforms to a straight line is a

measure of system linearity; **NOTE 3:** Linearity typically refers to the overall system response rather than the raw instrument output, but in this guideline, the definition applies to both and is considered especially pertinent to instrument output.

**measurand** – quantity intended to be measured (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 1:** The specification of a measurand requires knowledge of the kind of quantity, description of the state of the phenomenon, body, or substance carrying the quantity, including any relevant component, and the chemical entities involved (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 2:** In the second edition of the VIM and in IEC 60050-300:2001, the measurand is defined as the ‘quantity subject to measurement’ (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 3:** The measurement, including the measuring system and the conditions under which the measurement is carried out, might change the phenomenon, body, or substance such that the quantity being measured may differ from the measurand as defined. In this case, adequate correction is necessary (ISO/IEC Guide 99)<sup>13</sup>; **EXAMPLE 1:** The potential difference between the terminals of a battery may decrease when using a voltmeter with a significant internal conductance to perform the measurement. The open-circuit potential difference can be calculated from the internal resistances of the battery and the voltmeter (ISO/IEC Guide 99)<sup>13</sup>; **EXAMPLE 2:** The length of a steel rod in equilibrium with the ambient Celsius temperature of 23 °C will be different from the length at the specified temperature of 20 °C, which is the measurand. In this case, a correction is necessary (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 4:** In chemistry, “analyte,” or the name of a substance or compound, are terms sometimes used for ‘measurand.’ This usage is erroneous because these terms do not refer to quantities (ISO/IEC Guide 99).<sup>13</sup>

**measurement procedure** – detailed description of a measurement according to one or more measurement principles and to a given measurement method, based on a measurement model and including any calculation to obtain a measurement result (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 1:** A measurement procedure is usually documented in sufficient detail to enable an operator to perform a measurement (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 2:** A measurement procedure can include a statement concerning a target measurement uncertainty (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 3:** A measurement procedure is sometimes called a standard operating procedure, abbreviated SOP (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 4:** Formerly in this document, the term “analytical method” was used.

**precision (measurement)** – closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 1:** Measurement precision is usually expressed numerically by measures of imprecision, such as standard deviation, variance, or coefficient of variation under the specified conditions of measurement (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 2:** The ‘specified conditions’ can be, for example, repeatability conditions of measurement, intermediate precision conditions of measurement, or reproducibility conditions of measurement (see ISO 5725-1:1994)<sup>19</sup> (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 3:** Measurement precision is used to define measurement repeatability, intermediate measurement precision, and measurement reproducibility (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 4:** Sometimes, “measurement precision” is erroneously used to mean measurement accuracy (ISO/IEC Guide 99).<sup>13</sup>

**quality assurance (QA)** – part of quality management focused on providing confidence that quality requirements will be fulfilled (ISO 9000)<sup>20</sup>; **NOTE:** The term “quality assurance” refers to all procedures and activities directed toward ensuring that a specified quality of product is achieved and maintained. In the testing environment, this process includes monitoring raw materials, supplies, instruments, and procedures; sample collection/transport/storage/processing; recordkeeping; calibrating and maintaining equipment; conducting quality control procedures; proficiency testing; and training of personnel and all else involved in the production of the data reported.

**quality control (QC)** – the operational techniques and activities that are used to fulfill requirements for quality (modified from ISO 9000)<sup>20</sup>; **NOTE 1:** In health care testing, the set of procedures designed to monitor the test method and the results to ensure test system performance; **NOTE 2:** QC includes testing control materials, charting the results and analyzing them to identify sources of error, and evaluating and

documenting any remedial action taken as a result of this analysis; **NOTE 3:** In clinical laboratory testing, QC includes the procedures intended to monitor the performance of a test system to ensure reliable results.

**repeatability** – precision under conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time (ISO 5725-1:1994)<sup>19</sup>; **NOTE 1:** ISO 3534-1<sup>17</sup> defines repeatability as “precision under repeatability conditions”; **NOTE 2:** Formerly, the term within-run precision was used in CLSI documents.

**reproducibility** – precision under conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment (ISO 5725-1).<sup>19</sup>

**sample** – one or more parts taken from a system, and intended to provide information on the system, often to serve as a basis for decision on the system or its production (ISO 15189)<sup>21</sup>; **NOTE 1:** For example, a volume of serum taken from a larger volume of serum (ISO 15189)<sup>21</sup>; **NOTE 2:** A sample is prepared from the patient specimen and used to obtain information by means of a specific laboratory test; **NOTE 3:** In CLSI document GP02, the term “sample” replaces the term “specimen”; however, for the purposes of this guideline, readers can consider the terms equivalent; **NOTE 4:** The term “specimen” has been used in laboratory medicine as a synonym for a sample, as defined here, of biological origin, or for an entire macroscopic parasite; **NOTE 5:** The system from which a sample is taken may not be of the same type as that of the measurand. For example, a given blood sample may serve for measurement of pH in plasma hemoglobin concentration in erythrocytes.

**sensitivity (of a measuring system)** – quotient of the change in an indication of a measuring system and the corresponding change in a value of a quantity being measured (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 1:** Sensitivity of a measuring system can depend on the value of the quantity being measured (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 2:** The change considered in a value of a quantity being measured must be large compared with the resolution (ISO/IEC Guide 99).<sup>13</sup>

**specimen** – biological material which is obtained in order to detect or to measure one or more quantities, such as amount or concentration (ISO/TR 18112-1)<sup>22</sup>; **NOTE:** Examples include whole blood, urine, stool, cerebrospinal fluid, or solid tissues.

**steatorrhea** – the presence of excessive fecal fat.

**trueness (measurement)** – closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 1:** Measurement trueness is not a quantity and thus cannot be expressed numerically, but measures for closeness of agreement are given in ISO 5725<sup>19</sup> (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 2:** Measurement trueness is inversely related to systematic measurement error, but is not related to random measurement error (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 3:** Measurement accuracy should not be used for ‘measurement trueness’ and vice versa (ISO/IEC Guide 99).<sup>13</sup>

**verification** – provision of objective evidence that a given item fulfills specified requirements (ISO/IEC Guide 99)<sup>13</sup>; **EXAMPLE:** Confirmation that performance properties or legal requirements of a measuring system are achieved (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 1:** The item may be, eg, a process, measurement procedure, material, compound, or measuring system (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 2:** The specified requirements may be, eg, that a manufacturer’s specifications are met (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 3:** Verification should not be confused with calibration (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 4:** Not every verification is a validation (ISO/IEC Guide 99)<sup>13</sup>; **NOTE 5:** In chemistry, verification of the identity of

the entity involved, or of activity, requires a description of the structure or properties of that entity or activity (ISO/IEC Guide 99).<sup>13</sup>

**validation** – verification (provision of objective evidence that a given item fulfills specified requirements) where the specified requirements are adequate for an intended use, where the specified requirements are adequate for an intended use (ISO/IEC Guide 99)<sup>13</sup>; **EXAMPLE:** A measurement procedure, ordinarily used for the measurement of mass concentration of nitrogen in water, may be validated also for measurement in human serum (ISO/IEC Guide 99).<sup>13</sup>

### 3.3 Abbreviations and Acronyms

AMI	analytical measurement interval
CAP	College of American Pathologists
CF	cystic fibrosis
CFF	Cystic Fibrosis Foundation (North American)
CFTR	cystic fibrosis transmembrane conductance regulator
Cl <sup>-</sup>	sweat chloride
CV	coefficient of variation
ISE	ion-selective electrode
KID	keratitis, ichthyosis, deafness
LoQ	limit of quantitation
NaCl	sodium chloride
NEQAS	National External Quality Assessment Schemes (UK)
QA	quality assurance
QC	quality control
QNS	quantity not sufficient
RCPA-QAP	Royal College of Pathologists Australasia Quality Assurance Program
SD	standard deviation
SI	Système International d'Unités
TPI	triosephosphate isomerase
USP	US Pharmacopeia

## 4 Procedural Precautions

Use powder-free gloves when collecting and analyzing sweat, because powder can affect the weight measurements and contaminate the samples. Individuals with latex sensitivity should wear latex-free gloves.

### 4.1 Chemical Hygiene

Prepare all solutions requiring the use of concentrated acids and bases in accordance with all applicable federal guidelines. In addition, laboratory personnel should refer to the laboratory's chemical hygiene plan.

### 4.2 Verification of Measurement Procedures

Follow the applicable local and federal guidelines for verification of measurement procedures and instruments before patient testing using samples equivalent in volume and measurand (analyte) concentration to patient samples (see Section 9.7).

### 4.3 Burns

Burns to the patient's skin after iontophoresis are extremely rare. The burns can occur at either electrode and range from tiny, black dots the size of pinholes to larger, crater-like lesions. If a burn does occur, rinse the affected area well with distilled or deionized water, and seek appropriate medical attention. Do not collect sweat over the area of the burn. To minimize the possibility of a burn, observe the following steps:

#### 4.3.1 Cleanse the Skin

Cleanse the skin with an alcohol pad followed by distilled or deionized water before attaching the electrodes. This removes dead surface skin cells and hydrates the top skin layer. It also removes any contaminating lotions or creams that might interfere with the iontophoresis or subsequent analysis.

#### 4.3.2 Maintain a Wet Interface

Maintain a wet interface with the skin during iontophoresis.

(1) If using iontophoresis reagents in gel form

- Avoid prolonged exposure of the gel to the air. If the gel surface appears to have lost some of its wetness, apply a drop of distilled or deionized water to the skin or to the gel just before attaching the gel-filled electrode to the extremity.
- As an added precaution, leave the skin damp after cleansing and before iontophoresis.

(2) If using reagents on gauze or filter paper

- The gauze or filter paper must be thoroughly saturated with reagents during the iontophoresis.

#### 4.3.3 Limit Iontophoretic Current

Burn potential increases with the magnitude and duration of iontophoretic current density (see Appendix B). When using a manually controlled current source with a 2 × 2-in (5.1 × 5.1-cm) gauze pad, at the beginning of the iontophoresis, set the current limit for the lowest possible current of about 0.5 milliamperes (mA); then, bring the current up slowly to 2.5 to 4 mA. Do not exceed 4 mA. Maintain the current for five minutes. During the five minutes, monitor the current closely to ensure that it does not exceed 4 mA. Automatically controlled current sources should be programmed to accomplish such a ramped current profile.

#### 4.3.4 Avoid Skin-Electrode Contact

(1) If using iontophoresis reagents in gel form

Inspect gel discs for cracks or any structural defect that would allow direct skin-electrode contact. Discs exposed to air for a long time will lose water and shrink in size. Reject any disc with a diameter that is significantly smaller than that of the electrode. Do not use any disc that is internally fractured or that is crumbled as a result of freezing. The disc should fit snugly into the recess of the plastic electrode assembly.

(2) If using reagents on gauze or filter paper

To prevent the possibility of direct electrode contact with the skin, which will cause a burn, the gauze or filter paper should be slightly larger than the bare electrodes. Position the electrodes carefully on the gauze or filter paper so a generous margin of the gauze or filter paper extends from under each edge of the electrode.

#### **4.3.5 Attach Electrodes Firmly**

Always apply the electrodes using firm tension in the attachment straps to ensure that a uniform, low-resistance interface exists between the electrolyte reservoir and the skin. This minimizes patient discomfort and reduces the possibility of burn formation.

#### **4.3.6 Keep the Electrode Surfaces Clean**

It is important to keep the electrode surfaces clean and free of any surface oxidation that might increase electrical resistance between the electrode and the electrolyte reservoir. Emery cloth can be used to clean the electrodes. Do not use commercial abrasive metal cleaners because of the potential for introduction of toxic chemicals into the skin during iontophoresis. Additional electrode and equipment disinfection may be required based on the institution's infectious control practices (see Section 6). Do not use disinfectants like bleach that contain chloride, which can contaminate the sweat sample.

### **4.4 Electrical Malfunctions**

To minimize the risk to the patient, follow these guidelines:

- Use a battery-powered iontophoresis system. This avoids the possibility of subjecting the patient to line voltage in the event of a component failure.
- It is desirable for the iontophoresis system to have a transistor current-limiting circuit that limits the amount of current delivered.
- The iontophoresis system should undergo a documented, regular maintenance procedure by medical engineering personnel for voltage leak and current control. Maintenance frequency depends on institutional and other regulatory requirements.
- Iontophoresis should not be performed on a patient receiving oxygen by an open delivery system. While the possibility of an explosion owing to the generation of an electrical spark is remote, it cannot be ignored. Iontophoresis can be performed on patients receiving oxygen by face mask or nasal cannula.

### **4.5 Allergic Reactions**

Allergic reactions to pilocarpine iontophoresis are extremely rare.<sup>23</sup> If, however, after the iontophoresis and/or collection, an area of diffuse inflammation and urticaria (hives) occurs, immediately discontinue the procedure, seek appropriate medical attention, and do not assay the sweat.

Keep an anaphylaxis treatment kit in the area for use by trained personnel or ensure that an alternate plan for treatment in case of emergencies is in place. Store the kit and components at the appropriate temperature and ensure it includes the following items:

- 1-mg/mL epinephrine ampules
- 1-mL tuberculin syringes
- 50-mg diphenhydramine hydrochloride capsules
- 25-mg diphenhydramine hydrochloride elixir in 10-mL aliquots
- Alcohol preparation pads

## 5 Principle

The procedure involves

- Sweat stimulation
- Sweat collection
- Quantitative sweat measurement for chloride

Common clinical indications for measuring chloride in sweat include the following:

- A family history for CF
- Positive newborn screening test result
- Presence of two CF-causing mutations in the fetus
- *In utero* echogenic bowel: neonatal intestinal obstruction
- Steatorrhea
- Rectal prolapse
- Chronic cough
- Chronic wheezing
- Persistent or recurrent respiratory tract infections
- Nasal polyps
- Failure to thrive

However, it is important to consider the diagnosis of CF and perform such testing in patients with a wide variety of signs and symptoms (see Appendix A).

### 5.1 Sweat Stimulation

Localized sweating can be produced by the iontophoresis of the cholinergic drug pilocarpine nitrate into an area of skin. Iontophoresis uses a voltage so electrical current carries the ionized drug into the skin. The positively charged pilocarpine ions move away from the positive electrode and into the skin where they stimulate the sweat glands. A negative electrode with a dilute electrolyte solution is applied to the same limb. The patient should be closely supervised during iontophoresis.

To ensure sweat is collected from adequately stimulated sweat glands at an appropriate rate and electrolyte concentration, the sweat rate should exceed 1 g/m<sup>2</sup>/minute. This rate is ensured by adhering to the minimum sweat weight or volume described in Section 8.7. The stimulation and collection area must be the same size in order to standardize the sweat rate and minimize the evaporation or dilution of sweat chloride from unstimulated sweat.<sup>24</sup>

### 5.2 Sweat Collection

After stimulation (see Section 8.4), the skin is cleansed with distilled or deionized water and thoroughly dried. Sweat can then be collected by two different methods using gauze/filter paper or microbore tubing.

#### 5.2.1 Gauze or Filter Paper

Using absorbent pad collection, the preweighed gauze or filter paper is placed over the site of the positive electrode and covered with a paraffin wax film to prevent evaporation. After collection (see Section 8.4.1), the gauze or filter paper is removed and reweighed. The increase in the weight corresponds to the volume (mass/density) of the sweat collected. The relative density of sweat ranges from 1.001 to 1.008.<sup>25</sup> The sweat is then separated from the gauze or filter paper via elution (see Section 9.3), and an aliquot is used for chloride determination.<sup>26</sup>

### 5.2.2 Microbore Tubing

Sweat can also be collected into self-harvesting, transparent, flexible, microbore tubing (see Section 8.5.1). The collector consists of a slightly concave disc. A hole in the center of the disc admits a small, plastic microbore tube that is coupled to the disc and is coiled on the upper side of the disc. Sweat is forced through the central hole by hydrostatic pressure and is collected in the microbore tubing. A small amount of blue dye is painted on the bottom of the collecting disc, which allows for visualization of the collected sweat. After adequate specimen collection, the tube is closed off and cut free from the disc for the measurement of chloride.<sup>27,28</sup>

### 5.2.3 Collection Time

Sweat must be collected for no more than 30 minutes. Sweat stimulation occurs for a maximum of 30 minutes following iontophoresis. Extending the time beyond 30 minutes does not significantly increase the sweat yield, potentially allows for evaporation, and increases the minimum amount of sweat required for a rate of 1 g/m<sup>2</sup>/min.<sup>24</sup>

## 5.3 Measurement of Chloride in Sweat

### 5.3.1 Units of Measurement

This guideline uses Système International d'Unités (SI) and provides measurements in millimole per liter (mmol/L). For chloride, milliequivalent per liter (mEq/L) and millimole per liter (mmol/L) are equivalent because the valence of chloride is 1.

### 5.3.2 Recommended Methods

After an adequate amount of sweat is collected, it can be analyzed for chloride using coulometric titration with a chloridometer.

### 5.3.3 Other Methods for Sweat Chloride

Analytical instrumentation and methodology for sweat determination should be approved by appropriate regulatory agencies for use on sweat samples, or the methodology must be verified and validated internally (see CLSI documents EP05,<sup>29</sup> EP06,<sup>30</sup> EP07,<sup>31</sup> EP10,<sup>32</sup> and EP15<sup>33</sup> on Evaluation Protocols and Section 9.7). There are limited reports on the use of ion-selective electrode (ISE) measurements for sweat chloride; however, many analytical systems that employ ISE measurements of chloride in serum and other body fluids have not been validated systematically for sweat electrolyte determination.<sup>34-37</sup> If such systems are employed, the user must validate the methods against traditional quantitative procedures described in the document. There is a concern when using ISEs to measure sweat chloride that the sensitivity at the lower concentrations could compromise the accuracy and precision of the results.

Other manual chloride methods, such as Schales and Schales, can be used if validated.<sup>38</sup>

## 6 Apparatus and Equipment

All reusable equipment and apparatus that come in contact with the patient should be disinfected with each use according to the institution's infectious control policies and procedures. Equipment such as the iontophoresis power supply should be periodically disinfected. Disinfection procedures should differ according to whether the patient contact involved intact or nonintact skin (eg, burn). Do not use disinfectants like bleach that contain chloride, which can contaminate the sweat sample. Reusable equipment such as scissors, nippers, and sweat dispensers should be rinsed with distilled or deionized water and dried with each use to ensure they are not contaminated with sweat.

## 6.1 Iontophoresis Equipment

### 6.1.1 Current Source

If the current source is manually controlled, a milliammeter should be supplied that enables the operator to keep the current below 4 mA. For safety reasons, the current source should be battery-powered. Instruments designed with a voltage of 22.5 V are sufficient for iontophoresis involving sweat collection onto gauze or filter paper. Somewhat more voltage is used in automatically controlled units using smaller electrodes. When using a manually controlled current source with a 2 × 2-in (5.1 × 5.1-cm) gauze pad, at the beginning of the iontophoresis, set the current limit for the lowest possible current of about 0.5 milliamperes (mA); then, bring the current up slowly to 2.5 to 4 mA. Do not exceed 4 mA. Maintain the current for five minutes. On automatically controlled units, a low-battery indicator is helpful. Do not use current sources designed for use with gauze or filter paper with smaller pilocarpine gel discs or vice versa. With pilocarpine gel discs, use only the power supply designed for the discs. (Refer to Appendix B for a discussion of current density.)

### 6.1.2 Electrodes

The electrodes for collection of sweat are made of copper or stainless steel and have central posts or some other device that allows the attachment of straps. The electrode surface should be smooth, and free of irregularities and surface oxidation.

#### 6.1.2.1 For Gauze or Filter Paper Collection

To standardize the sweat minimum sample requirement, it is recommended that the same size electrode be used for all ages of patients. The stimulated area should be 2 × 2 in (total area 4 square in) or 5.1 × 5.1 cm (total area 26.01 square cm). A slightly smaller electrode (eg, 1.5 × 1.5 in or 3.75 × 3.75 cm) is used for iontophoresis. Other electrode sizes are acceptable if they cover greater than 50% of the 2 × 2 in area (ie, an area of greater than 2 square in). After stimulation, the sample should be collected from a single site using 2 × 2-in (5.1 × 5.1-cm) gauze or filter paper.<sup>24</sup>

#### 6.1.2.2 For Microbore Tubing Collection

Electrodes for pilocarpine gel discs are commercially available that provide a snug fit for the gel disc into the electrode assembly and correspond in size to the microbore tubing collector.

### 6.1.3 Electrode Straps

Use suitable straps (eg, nonlatex elastic or hook-in-loop fabric) to secure the electrodes on the patient's collection site.

### 6.1.4 Analytical Balance

An analytical balance used to weigh the amount of sweat collected should be sensitive to 0.0001 g.

### 6.1.5 Scissors

If sweat is collected in gauze or filter paper, scissors are used to cut the paraffin wax film.

### 6.1.6 Nippers

If the sweat is collected in microbore tubing, nippers are used. They are supplied by the manufacturer of the collection system.

### 6.1.7 Sweat Dispenser or Tuberculin Syringe

If the sweat is collected in microbore tubing, sweat dispensers or tuberculin syringes are used. The sweat dispenser is supplied by the manufacturer of the collection system.

## 6.2 Chloridometer

Use a chloridometer to determine sweat chloride concentration. Additional supplies such as titration vials may be required. If the vials are reused, ensure there is no carryover or contamination between samples.

## 7 Materials and Reagents

### 7.1 Iontophoresis Materials

#### 7.1.1 Water

Freshly prepared distilled or deionized water should be used. "Pure" or sterile water may not be distilled or deionized and should not be used. Refer to CLSI document C03<sup>39</sup> for water specifications.

#### 7.1.2 Pilocarpine Nitrate Solution

A 7.4 to 18.4 mmol/L (2 to 5 g/L; 0.2 to 0.5% w/v) solution of pilocarpine nitrate is used (see Appendix C). The source of the pilocarpine nitrate should be US Pharmacopeia (USP) ([www.usp.org](http://www.usp.org)) grade or equivalent. Many laboratories use a 14.7 mmol/L (4 g/L; 0.4% w/v) solution of pilocarpine. This is prepared by dissolving 1.2 g of pilocarpine nitrate in 300 mL of distilled or deionized water. This solution is stable for three months at 4 °C and one month at room temperature.

Pilocarpine nitrate is also available in an 18.4 mmol/L (5 g/L; 0.5% w/v) concentration gel disc for use with the microbore tubing collectors. The gel discs are stored refrigerated at temperatures from 2 °C to 8 °C and are stable until the expiration date indicated by the manufacturer. Do not freeze.

#### 7.1.3 Electrolyte Solution

A dilute electrolyte solution is required for the negative electrode (cathode). In order to negate the possibility of contamination of the sweat sample with extraneous chloride from an electrolyte solution, do not use saline. A solution of 0.05 mol/L magnesium sulfate (MgSO<sub>4</sub>) or 0.01 mol/L sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) is recommended.

A 0.05 mol/L MgSO<sub>4</sub> USP grade or equivalent solution is prepared by dissolving 12.4 g MgSO<sub>4</sub> • 7H<sub>2</sub>O in a sufficient volume of distilled or deionized water to bring the total volume to 1 L. The solution is stable for six months at room temperature.

A 0.01 mol/L H<sub>2</sub>SO<sub>4</sub> solution is prepared by adding 0.56 mL of concentrated sulfuric acid to 500 mL of distilled or deionized water in a liter volumetric flask and diluting to 1 L with distilled or deionized water. This is stable for six months at room temperature.

With the microbore tubing collection, a second pilocarpine nitrate gel disc is used at the negative electrode.

#### 7.1.4 Gauze Pads and Tissues

Use gauze or laboratory tissues to clean and dry the patient's skin.

### 7.1.5 Gauze and Filter Paper

Use gauze squares or filter paper as the absorbent pad for the iontophoresis reagents. Use the same size gauze or filter paper (2 × 2 in [5.1 × 5.1 cm]) for stimulation and collection.

### 7.1.6 Emery Cloth

Emery cloth should be available for cleaning the electrodes.

### 7.1.7 Anaphylaxis Treatment Kit

See Section 4.5.

## 7.2 Sweat Collection Supplies

The following supplies are needed to perform sweat testing on gauze or filter paper, or in microbore tubing.

### 7.2.1 On Gauze or Filter Paper

- Gauze squares or filter paper for stimulation and collection: These should be low in sodium and chloride content. If filter paper is used, it should be of a type sufficiently absorbent to collect all of the stimulated sweat. Use the same size gauze or filter paper (2 × 2 in [5.1 × 5.1 cm]; total area 4 square in [26.01 square cm]) for stimulation and collection.
- Weighing vials: Disposable plastic or glass vials with a snugly fitting cap should be sufficient to hold 10 mL of diluent. If the weighing vials are reused, ensure they are well washed and rinsed with distilled or deionized water and thoroughly dried to avoid contamination of the sweat sample.
- Two pieces of paraffin wax film cut into squares measuring 7.6 cm (3.0 in).
- Waterproof surgical tape [2.5 cm (1.0 in) wide] or disposable latex-free stretch bandage and paper tape.
- Powder-free gloves.

### 7.2.2 In Microbore Tubing

- Sweat collector with attachment strap
- Powder-free gloves
- 0.2-mL polypropylene microcentrifuge tubes with snugly fitting caps
- Disposable latex-free stretch bandage (optional)
- Collector volume calibration chart (supplied by the manufacturer)

## 7.3 Chloride Determination by Chloridometer

- Acid diluent; acetic acid/nitric acid: 250 mL glacial acetic acid and 16 mL concentrated nitric acid. Add 250 mL of glacial acetic acid and 16 mL of concentrated nitric acid to 1000 mL of distilled or

deionized water, and mix thoroughly. Store in a glass bottle. The diluent is stable for six months at room temperature.

- Gelatin reagent: Gelatin reagent can be obtained commercially in a preparation containing a pH indicator. Follow the manufacturer's recommendations for preparation and storage.

**NOTE:** Commercially prepared acid reagent containing polyvinyl alcohol is available. If this reagent is used, gelatin reagent is not needed.

- Chloride calibrators, 1.0 mmol/L and 100 mmol/L: The chloride calibrators can be prepared in-house or purchased commercially.

To prepare a 1.0-mmol/L chloride calibrator, weigh 0.0584 g of desiccated sodium chloride (NaCl) and add sufficient volume of distilled or deionized water to bring the volume to 1 L.

To prepare a 100-mmol/L chloride calibrator, weigh 5.8441 g of desiccated NaCl and add sufficient volume of distilled or deionized water to bring the volume to 1 L.

Document the verification of newly prepared calibrators against existing calibrators.

Store the calibrators in a tightly stoppered glass bottle at 4 °C. The calibrators are stable for three months or according to the manufacturer's recommendations. Bring them to room temperature before use.

- Low- and high-chloride controls: Recommended concentration for the low control is in the range of 10 to 30 mmol/L with a high control greater than or equal to 100 mmol/L chloride. Prepare the controls separately from the calibrators used to calibrate the chloridometer.
- The chloride controls may be prepared in-house or purchased commercially. To prepare a 10-mmol/L chloride control, weigh 0.5844 g of desiccated NaCl and add a sufficient volume of distilled or deionized water to bring the volume to 1 L.

To prepare a 100-mmol/L chloride control, weigh 5.8441 g of desiccated NaCl and add a sufficient volume of distilled or deionized water to bring the volume to 1 L.

- Store controls in tightly stoppered glass bottles at 4 °C or as recommended by the manufacturer. The controls are stable for three months or according to the manufacturer's recommendations. Bring them to room temperature before use.

To assign control values to commercial controls, assay the controls once a day for 20 days and calculate the mean and standard deviations (SD). For controls that are prepared gravimetrically in-house, use the weighed in target value and calculate the SD (see CLSI document EP05).<sup>29</sup>

## 8 Sweat Stimulation and Collection Procedures

### 8.1 Patient Education

Written, computerized, or video format patient educational materials should be provided. The materials should include indications for testing, an overview of the procedure and the time involvement, any risks such as burns, the potential for retesting (ie, if a sufficient amount of sweat is not obtained; or if the results are intermediate; or inconsistent with the clinical features), and how and when the results will be communicated. A patient educational DVD is available in English and Spanish.<sup>40</sup>

## 8.2 Effects of Patient Age

Clinical experience indicates that it can be more difficult to obtain an adequate sweat sample during the first four weeks after birth. This can be a particular problem among preterm infants. To increase the likelihood of collecting an adequate sweat specimen, it is recommended that sweat chloride testing in asymptomatic newborns with a positive newborn screen result first be performed when the infant is at least 2 weeks of age and weighs >2 kg. Earlier testing could lead to misleading results, because sweat chloride concentrations in healthy newborns gradually decrease during the first weeks of life. In symptomatic newborns (eg, those with meconium ileus), sweat chloride can be evaluated as early as 48 hours after birth if an adequate sweat volume can be collected.<sup>2</sup>

## 8.3 Collection Timing and Sites

Ideally, collection of sweat should be carried out at a time when the patient is clinically stable, well-hydrated, free of acute illness, and not receiving mineralocorticoids. There is no information regarding the effect of corticosteroids on sweat electrolyte concentration. Repeat sweat collection and analysis can be carried out at any time after the initial testing. For example, if a patient was sweat tested with bilateral collections in the morning and failed to produce an adequate sample, the patient could be retested once again that same day using a different site for stimulation and collection.

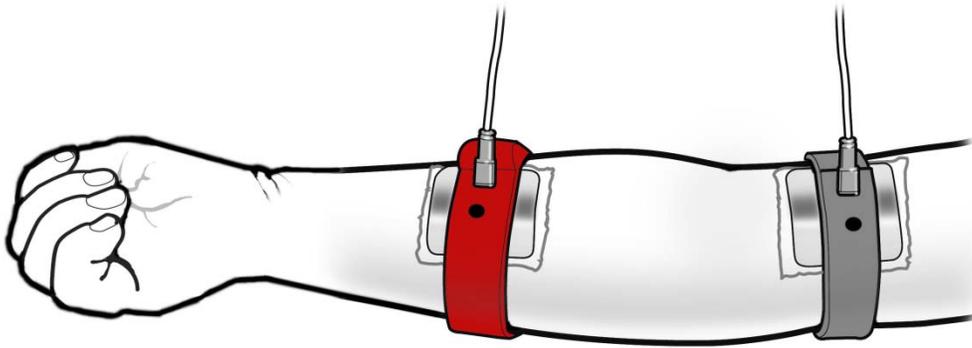
Sweat can be collected from a site receiving intravenous fluids as long as good contact between the skin and the electrode is possible, and collection techniques do not interfere with venous flow. Sweat can be collected, with equivalent electrolyte concentration, from the lower arm or thigh; although the density of sweat glands is less in the thigh, making it a less optimal collection site.<sup>41</sup> If the leg is used, the positive electrode should be placed in the inner thigh and the negative electrode on the calf. Before stimulation, cleanse the area of any urine contamination, and during collection, ensure that the site does not become contaminated with urine.

Do *not* stimulate or collect sweat from the following sites:

- Head, including forehead (possible burns)
- Trunk (current crossing heart)
- Any area of inflammation (eg, eczema or rash); serous or bloody discharge (contamination)<sup>42</sup>

### 8.3.1 Using Gauze/Filter Paper

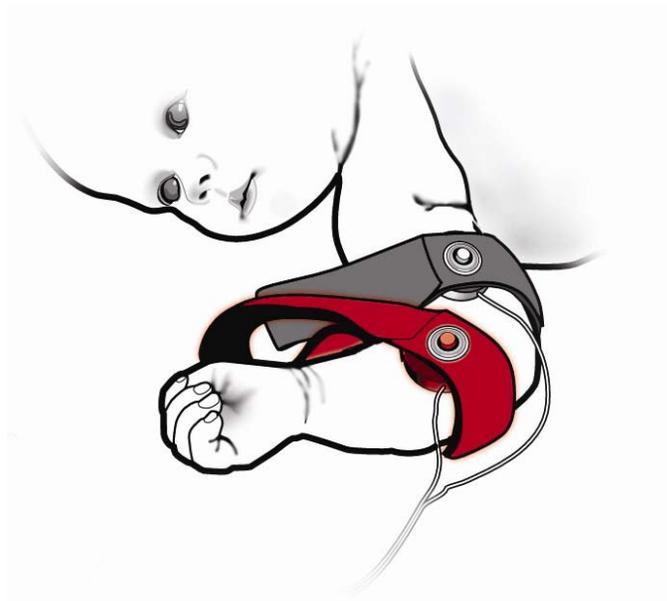
On the arm, the negative electrode should be placed half-way between the shoulder and elbow on the inner surface of the upper arm, and the positive electrode (subsequent collection site) should be placed on the inner volar surface of the forearm half-way between the elbow and wrist (see Figure 1). Note that the positive electrode should not be too close to the wrist to avoid problems in sweat collection caused by tendon and wrist flexing during the collection period.



**Figure 1. Placement of Electrodes for Gauze or Filter Paper Collection**

### 8.3.2 Using Microbore Tubing

If the sweat is collected into microbore tubing, the collection device should not be placed so close to the wrist that the tendons are palpable, because reasonably thick musculature is required to properly seal the collector against the skin (see Figure 2). In addition, if the thigh is selected as the collection site, restraining the patient's leg motion to control flexing of the upper leg muscles will help ensure successful collection.



**Figure 2. Placement of Electrodes With Microbore Tubing Collector**

## 8.4 Stimulation and Collection of Sweat on Gauze or Filter Paper

### 8.4.1 Gauze or Filter Paper Stimulation and Collection Procedure

- (1) No portion of the collection materials, collection site, or weighing vials should directly contact the hands. Use powder-free gloves.
- (2) Place a piece of gauze in a plastic or glass vial. Securely fasten the plastic cap.

- (3) Label the cap and body of the weighing vial with patient information or unique vial identification and the designation “left” and “right” before determining the initial weight (see Section 11.2). Nothing should be written or attached to the weighing vial after it is initially weighed.
- (4) Zero the analytical balance. Weigh the labeled vial containing the gauze on an analytical balance. Record the weight on a worksheet.
- (5) Place the preweighed vial in a desiccator for storage. Once the vial is removed from the desiccator, it should be placed inside a clean, powder-free carrier glove or resealable plastic bag for transport. The gauze should be placed on the patient within 30 minutes. In certain circumstances such as sending the vials to an off-site facility for sweat collection, it may not be possible to use vials within 30 minutes after removing the vials from the desiccator. In such cases, the vial can be placed in a resealable bag containing a packet of desiccant. To avoid change in the weight of the vial, it is important not to use powder or grated desiccant. Each laboratory should validate the storage and transport conditions to make sure the weight of vial does not change with time.
- (6) For convenience, several vials may be labeled, weighed, and placed in the desiccator for daily storage.
- (7) Before administering the test, ensure the battery charge is sufficient for the testing or follow the manufacturer’s guidelines.
- (8) Check that the electrodes are clean, shiny, and free of irregularities. With each use, clean copper electrodes with emery cloth, rinse them with distilled or deionized water, and dry them with gauze.
- (9) Wash the patient’s stimulation sites well using an alcohol pad followed by a gauze pad soaked in distilled or deionized water. Dry the skin thoroughly with gauze pads.
- (10) Select or cut the appropriate size of gauze (see Sections 6.1.2.1 and 7.1.5). Saturate one piece of the gauze with pilocarpine nitrate (see Section 7.1.2), and saturate another piece with the dilute electrolyte solution not containing chloride (see Section 7.1.3). To avoid bridging between the electrodes, the gauze pads should be thoroughly moistened but not dripping.
- (11) Place the gauze saturated with the dilute electrolyte solution on the selected site, and secure the plate of the negative electrode over the gauze using the supplied straps. Place the pilocarpine-saturated gauze on the collection site, and secure the plate of the positive electrode over the gauze using the supplied straps. Connect the electrodes to the iontophoresis power supply, and check that the pilocarpine site is attached to the positive electrode and positive pole of the power supply. Pilocarpine will not be delivered from the negative electrode.
- (12) Check to make sure the electrodes do not directly touch bare skin. Direct contact between electrodes and skin will result in burns.
- (13) Turn the power on. Set the current to the lowest value and slowly increase the current to 2.5 to 4 mA and maintain it at this level for five minutes. Never exceed 4 mA, because burns can occur. If the patient complains or shows signs of discomfort, this could be because of uneven distribution of current. If this occurs, check and possibly tighten the electrode strap to ensure even contact between the skin, the gauze pad, and the electrode.

- (14) During the five-minute iontophoresis period, prepare the following items:
  - (a) Cut two pieces of a paraffin wax film larger than the collecting gauze. Lay them aside with the protective backing down.
  - (b) Saturate several pieces of gauze with distilled or deionized water.
- (15) After the five-minute iontophoresis period, slowly decrease the current to 0 mA and turn off the power. Disconnect the leads and electrodes from the patient and remove the gauze. There is usually a slight reddening of the skin at the site of the iontophoresis.
- (16) Using several gauze pads saturated with distilled or deionized water, thoroughly cleanse the skin that was under the negative electrode. Dry the skin thoroughly with dry, clean gauze, and with a fresh supply of pads, repeat the cleaning and drying process on the skin that was under the positive electrode. This area will become the collection site. Failure to dry completely the collection area can lead to false-negative results because of dilution of the collected sweat. The collection site must not be touched with ungloved fingers.
- (17) If there is an area of severe inflammation appearing as either a burn or urticaria (hives), seek medical attention. Sweat should not be collected over areas of severe inflammation.
- (18) Remove the protective backing from the paraffin wax film. Lay the first piece of the paraffin wax film with the uncontaminated side up on a clean, dry paper towel. Similarly, overlay the first piece with the second piece of paraffin wax film, uncontaminated side up. This will ensure that a clean surface comes into contact with the patient's skin. Keeping the preweighed vial in the carrier glove or resealable plastic bag, carefully remove the collecting gauze from the preweighed vial and place it on top of the paraffin wax film. Place the paraffin wax film and gauze over the site of the pilocarpine iontophoresis, with the gauze contacting the skin.
- (19) Secure the paraffin wax film with two strips of waterproof tape on all sides or wrap the site with a disposable stretch bandage and secure it with paper tape. Create an airtight seal between the skin, gauze, and paraffin wax film sheets.
- (20) Record the time for completion of collection and note this on the laboratory requisition.
- (21) Repeat the entire procedure on the patient's opposite extremity, using the same size electrodes on both sites.
- (22) Allow the sweat to collect for 30 minutes. Extending the collection time will not significantly increase the sweat yield and can potentially lead to false results.
- (23) At the end of the 30 minutes, remove and discard the disposable stretch bandage or waterproof tape.
- (24) Gently blot the paraffin wax film against the gauze to collect any condensate that could have formed on the lower surface of the paraffin wax film during collection. Failure to collect this condensate can result in false-positive values.
- (25) Lift the corners of the paraffin wax film and carefully remove the paraffin wax film and gauze together. Do not touch the gauze with the gloves; rather, use the paraffin wax film as the gauze cover.
- (26) Quickly (to minimize evaporation) transfer the gauze into the vial from which it was removed; guide the gauze with the paraffin wax film. Do not place the paraffin wax film in the vial with the

gauze; discard the paraffin wax film after using it to guide the gauze into the vial. The gauze, the paraffin wax film, and the preweighed vial must never come into contact with the hands or be contaminated in any way. Always use powder-free gloves when handling the collection materials. Always keep the preweighed vial and lid inside the carrier glove or resealable plastic bag.

- (27) Reweigh the vial promptly and record the weight. At least 0.075 g of sweat should be collected using  $5.1 \times 5.1$ -cm ( $2 \times 2$ -in) gauze. To ensure analytical consistency, the same trained laboratory personnel who preweighed the vial should reweigh the vial on the same balance after sweat collection.

#### 8.4.2 Procedural Notes and Precautions

Refer to Section 13.7 for possible sources of error.

- (1) In place of wearing powder-free gloves to avoid contamination, the weighing vial, collecting gauze, and paraffin wax film can be handled with forceps that have been rinsed with distilled or deionized water and dried.
- (2) Filter paper can be used in place of gauze for stimulating and collecting the sweat.
- (3) To minimize evaporation of the sweat sample
  - Use two strips of waterproof adhesive tape on all sides of the paraffin wax film, or wrap with a disposable stretch bandage to produce an airtight seal.
  - After collection, quickly transfer the gauze or filter paper to the weighing vial and promptly reweigh the vial.
- (4) To minimize contamination
  - Use gauze and/or filter paper that is low in sodium and chloride content.
  - Wash and dry the patient's skin thoroughly.
  - Do not directly handle the weighing vial, the paraffin wax film, the collection site, or any of the collection materials with the fingers. Always use forceps or powder-free gloves.

### 8.5 Microbore Tubing Collector Stimulation and Collection Procedure

Follow the manufacturer's instructions for use of the collection system.

#### 8.5.1 Microbore Tubing Collector Stimulation and Collection Procedure

Refer to Section 13.7 for possible sources of error.

- (1) Carefully inspect the pilocarpine-containing gel discs to ensure there are no physical defects.
- (2) Microbore tubing collectors are individually wrapped. Keep the collector in the wrapper until it is placed on the patient's skin. To minimize the potential for contamination, avoid touching the concave collecting surface.

- (3) Ensure that the extremity is thoroughly cleansed with an alcohol pad followed by distilled, deionized water before iontophoresis. Following iontophoresis, cleanse the collecting area with distilled, deionized water and dry thoroughly before applying the microbore tubing collector.
- (4) To ensure an efficient sweat collection, fasten the microbore tubing collector to the extremity with firm strap pressure. Test for proper attachment after sweat appears in the tubing. (Refer to the manufacturer's instructions.)
- (5) **Do not attempt to remove the entire collector assembly from the patient's extremity before sealing an end of the tubing and separating the microbore tubing from the main body.** Transient vacuum can cause a loss of part or all of the specimen.
- (6) Sweat should be collected for 30 minutes. The minimum sample volume collected should be 15  $\mu$ L. The sample volume can be estimated using the manufacturer's collection guide and verified volumetrically (see Section 9.4), or gravimetrically. If gravimetrically, the labeled containers must be weighed initially on an analytical balance and weighed again after the sweat sample is added. Wear powder-free gloves when handling the specimen containers to avoid adding spurious weight. The preweighed specimen containers can be placed in a desiccator for storage. Once the container is removed from the desiccator, it should be placed inside a clean, powder-free carrier glove or resealable plastic bag for transport. In certain circumstances such as sending the vials to an off-site facility for sweat collection, it may not be possible to use vials within 30 minutes after removing the vials from the desiccator. In such cases, the vial can be placed in a resealable bag containing a packet of desiccant. To avoid change in the weight of the vial, it is important not to use powder or grated desiccant. Each laboratory should validate the storage and transport conditions to ensure the weight of the vial does not change with time.
- (7) For QA, it is recommended that the procedure be repeated by collecting and analyzing a sample from the opposite extremity.
- (8) Reusable equipment such as scissors, nippers, and sweat dispensers should be rinsed with distilled or deionized water and dried with each use to ensure they are not contaminated with sweat.

## 8.6 Procedural Notes and Precautions for Stimulation and Collection

- (1) Do not iontophorese both extremities simultaneously. Iontophorese one site, apply the collection material, and during the 30-minute collection time, stimulate and collect sweat on the other extremity. To ensure consistency of collection, the entire collection procedure should be performed by the same trained laboratory personnel.
- (2) The sweat collection device must be designed for use with the appropriate iontophoresis system so the stimulation and collection areas are equivalent and the appropriate minimum acceptable sweat volume or weight can be achieved.
  - (a) Examples of acceptable combinations include
    - Iontophoresis with pilocarpine gel and collection into microbore tubing.
    - Iontophoresis with copper/stainless steel electrodes over gauze/filter paper pilocarpine-saturated pads and collection into gauze/filter paper.
  - (b) Examples of unacceptable combinations include

- Iontophoresis with pilocarpine gel and collection into gauze/filter paper. Do not use current sources designed for use with gauze or filter paper with smaller gel discs or vice versa. With gel discs, use only the power supply designed for the discs.
  - Stimulation with copper/stainless steel electrodes over gauze/filter paper pilocarpine-saturated pads and collection into microbore tubing.
- (c) Any other hybrid combinations.
- (3) The practice of bilateral testing (ie, collecting and analyzing sweat from two sites) may be especially useful, not only for QA, but in situations in which an insufficient sample may be more likely (for example, in testing infants). Although samples from separate sites may not be pooled for analysis, testing bilaterally increases the likelihood of collecting a sufficient sample from at least one site.<sup>2</sup>

## 8.7 Sample Requirements

Because sweat electrolyte concentration is affected by the rate of sweating, an accurate sweat test requires the measurement of sweat electrolytes from maximally stimulated sweat glands.<sup>43</sup> Measuring sweat electrolytes at low sweat rate could lead to false results.<sup>43</sup> In addition, evaporation becomes a more significant problem with smaller samples.<sup>24</sup>

The minimum acceptable sweat volume and/or weight depends on the size of the electrode used; the type and size of the collecting material used (gauze, filter paper, or microbore tubing); the length of time the sweat is collected; and the density of the sweat glands. 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. The sweat rate should exceed  $1\text{g}/\text{m}^2/\text{min}$ , which technically, corresponds to a minimum sample weight of  $0.077\text{ g}$  ( $77\text{ mg}$ ) of sweat collected on  $5.1 \times 5.1\text{-cm}$  ( $2.0 \times 2.0\text{-in}$ ) gauze or filter paper and  $18\text{ }\mu\text{L}$  of sweat collected in microbore tubing in 30 minutes. However, for many years,  $0.075\text{ g}$  ( $75\text{ mg}$ ) using gauze or filter paper and  $15\text{ }\mu\text{L}$  using microbore tubing has been used as the minimum acceptable sample amounts and there is no evidence that this has had an effect on the diagnostic validity of sweat chloride concentrations. Samples less than  $0.075\text{ g}$  ( $75\text{ mg}$ ) or  $15\text{ }\mu\text{L}$  should not be analyzed, nor should insufficient sweat samples be pooled to achieve the weight/volume requirement. It is imperative that the minimum sample amount be based on a specific collection time derived from the rate equation. For example, if a laboratory extends the collection time beyond 30 minutes, which is not recommended, a larger volume of sweat will be required.

In patients from whom an adequate sweat sample is not obtained, repeat testing should be carried out as soon as is practical. This could be the same day or the following day, because the rate of sweating can vary from day to day. At this time, there are no studies demonstrating the benefit of heating the collection site before iontophoresis or during sweat collection for the purpose of increasing the sweat yield. In cases in which adequate sweat samples cannot be obtained from initial bilateral testing, the test can be repeated again the same day at another site, but inadequate samples from several sites cannot be pooled for analysis.

### 8.7.1 Definition of Insufficient Sample

The amount of sweat collected should routinely be monitored to determine the proportion of patients from whom adequate sweat collection cannot be obtained. This proportion varies depending on the patient population. An annual target of less than 5% insufficient samples (aka quantity not sufficient [QNS]) for patients older than 3 months of age is recommended.<sup>2</sup> With bilateral collection, the test is considered QNS only if an adequate sweat sample is not obtained from either site. If the rate is greater than 5%, the cause should be investigated.

Several factors affect the ability to collect a sufficient sample, including the patient's age, weight, race, and the collection system used. For example, infants weighing less than 2 kg, younger than 38 weeks of age at testing, or of African American race have an increased likelihood of producing an insufficient sample.<sup>44</sup> Higher failure rates with the microbore tubing coil compared to gauze collection have been reported.<sup>27</sup>

### 8.7.2 Suggestions for Successful Sweat Collection

- The patient should be well hydrated and free of acute illness.
- When clinically acceptable, delay sweat testing until the infant is at least 2 weeks of age and weighs more than 2 kg.
- Check the polarity of the electrodes. Pilocarpine will not be delivered from the negative electrode.
- Ensure that the collection area corresponds to the site of iontophoresis. Sweat production is localized to glands under the positive (pilocarpine) electrode.
- Check that the pilocarpine is within the expiration date.
- To avoid bridging between the electrodes, check that the skin between the electrodes remains dry during iontophoresis. If the skin is wet with pilocarpine or electrolyte solution between the two electrodes, the current will “bridge” between them in a path of low resistance, and pilocarpine will not be transported into the sweat glands.

## 9 Analysis of Sweat Chloride by Titration With a Chloridometer

### 9.1 Principle

The chloride concentration in sweat can be determined from a sample by titration using a chloridometer. Sweat samples collected on gauze or filter paper require elution with a diluent before analysis. The following is one of several acceptable procedures for performing chloride measurement using a chloridometer. Analytical instrumentation and methodology for sweat determination should be approved by appropriate regulatory agencies for use on sweat samples, or the methodology must be validated internally (see CLSI documents EP05,<sup>29</sup> EP06,<sup>30</sup> EP07,<sup>31</sup> EP10,<sup>32</sup> and EP15<sup>33</sup> on Evaluation Protocols and Section 9.7).

### 9.2 Specimen Stability and Storage

It is important for laboratories to perform the sweat collection and analysis on the same day, and to report the results and interpretation to clinicians expeditiously. Standard procedure should be to perform the analysis shortly after collection, or within a few hours.<sup>2</sup> If, however, there is a significant delay between collection and analysis, the laboratory may store specimens using the guidelines below. The laboratory must validate any storage conditions other than those described.

#### 9.2.1 Collected on Gauze or Filter Paper

Sweat collected on gauze, once reweighed and secured in a vial with a tightly fitting lid, has been shown to be stable for up to 72 hours at refrigerator temperature.<sup>45</sup>

### 9.2.2 Collected Into Microbore Tubing

Users of microbore tubing systems are advised not to store or transport collected sweat in the tubing, as evaporation can begin to affect the results in as little as four hours at higher storage temperatures. If it is necessary to store or transport the samples for analysis, 0.2-mL microcentrifuge tubes with snugly fitting caps have been found to preserve the samples for 72 hours across a reasonable temperature range without significant evaporation.<sup>46</sup>

### 9.3 Procedure Using Gauze or Filter Paper Collection

- (1) Rinse with distilled or deionized water and dry several 1-mL volumetric pipettes or use disposable pipettes.
- (2) Place a piece of gauze the same size as the collecting gauze into a clean, plastic or glass vial with a cap. Label this vial "Gauze or Filter Paper Blank." The source for this gauze (or filter paper) should be the same as that used for the sample collection.
- (3) Prepare "control" vials. Weigh two vials (labeled "Low Control" and "High Control") that contain gauze or filter paper and record their weights. Add 100  $\mu\text{L}$  of the controls to the appropriately labeled vials. Reweigh the vials, record the weights, and subtract the difference. This difference should be  $0.1000 \text{ g} \pm 2\%$  ( $\pm 0.0020 \text{ g}$ ). If the tolerance is exceeded, the accuracy of pipettes and weighing should be investigated.
- (4) Using an automatic pipettor, add 8 mL of distilled or deionized water to the patient vials, the gauze or filter paper blank vial, and the control vials. Recap the vials and shake them for one minute. Allow the vials to sit at room temperature for at least 15 minutes. If sweat is collected on filter paper, the vials should not be shaken and the elution time should be increased to 40 minutes. Centrifuge to remove any filter paper fibers and use the supernatant for analysis.
- (5) At the end of the 15 minutes, use a pipette to press the sweat/diluent eluate from the gauze or filter paper and remove the gauze from the vials. Use the eluate as the test solution in Step 6.
- (6) Set up the chloride titration vials in duplicate as shown in Table 1 below, or follow the manufacturer's instructions. Individually label each titration vial. The solution's acidity is indicated by the presence of a pink color when four drops of gelatin reagent are added to the diluted sample. Do not titrate unless the pink color is present. Omit the gelatin reagent if using a commercially available acid reagent containing polyvinyl alcohol.

**Table 1. Chloride Titration Vials**

	<b>Blank</b>	<b>Gauze/Filter Paper Blank</b>	<b>Calibrator</b>	<b>Patient/Control</b>
Acid diluent	3 mL	3 mL	3 mL	3 mL
Distilled or deionized water	1 mL	—	—	—
1.0 mmol/L $\text{Cl}^-$ calibrator	—	—	1 mL	—
Gelatin reagent	4 drops	4 drops	4 drops	4 drops
Test solution from vials	—	1 mL	—	1 mL

- (7) For chloridometer set-up and analysis, follow the manufacturer's instructions and titrate the blanks, calibrator, and test/controls. Record the titration values.

- (8) Calculate the average of the duplicate readings for each vial; then, perform the following calculation:

$$\frac{\text{Test} - \text{Gauze Blank}}{\text{Calibrator} - \text{Blank}} \cdot \frac{\text{mL of Diluent (8 mL)} + \text{g of Sweat}}{\text{g of Sweat}} \cdot 1.0 \text{ mmol/L (Calibration Concentration)} = \text{mmol/L Sweat Cl}^-$$

Table 2 and the following equations are examples of how this calculation is performed:

**Table 2. Example Data**

	<b>Data</b>	<b>Mean</b>
Calibrator	140/142	141
Blank	40/44	42
Test	58/62	60
Gauze blank	49/49	49
Gram of sweat	0.1004	

$$\frac{60 - 49}{141 - 42} \cdot \frac{8 + 0.1004}{0.1004} \cdot 1 = \text{mmol/L Sweat Cl}^-;$$

$$\frac{11}{99} \cdot \frac{8.1004}{0.1004} \cdot 1 = 9 \text{ mmol/L Sweat Cl}^-.$$

#### 9.4 Procedure Using Microbore Tubing Collection

- (1) Chloride analysis is performed on 10  $\mu\text{L}$  of sweat collected in microbore tubing; however, the minimum amount of sweat that must be collected in a 30-minute period is 15  $\mu\text{L}$ .
- (2) Verify the amount of sweat collected, either gravimetrically or volumetrically. If volumetrically, use an adjustable 50- or 100- $\mu\text{L}$  pipette with a clean disposable tip. Set the volume on the pipette with the estimated volume of sweat. Aspirate sweat into the tip of the pipette. Dispense sweat back into the storage container. If sweat remains in the container after aspiration, adjust the volume up and repeat the procedure. If air is pipetted, adjust the volume down and repeat the procedure. Continue adjusting up or down until an accurate volume determination can be made. Record the volume collected.

For gravimetric determination of sweat volume, refer to Section 8.5.1 (6).

- (3) Use a serological pipette to transfer 4.0 mL of the acid diluent into individually labeled titration vials. Several blank vials are needed as well as vials for the 100-mmol/L calibrator, controls, and patient(s). Perform the titrations in duplicate when sufficient sample is available. Add four drops of gelatin reagent to each of the vials. Omit the gelatin reagent if a commercially prepared acid reagent is used that contains polyvinyl alcohol.
- (4) To the calibrator, controls, and patient titration vials, add 10  $\mu\text{L}$  of the appropriate sample.
- (5) Titrate each blank vial according to the manufacturer's recommendation. The reagent blank should titrate between 30 to 99 mmol/L. Calculate an average from the readings. Enter this value using the manufacturer's blank adjust feature. This value will be subtracted from all subsequent readings; thus, no further calculations are required.

If the blank concentration is less than 30 mmol/L using the internally prepared acid reagent described in Section 7.3, use a commercially prepared acid reagent.

If the blank concentration is greater than 99 mmol/L, repeat the blank vial titrations with freshly prepared acid reagent, or an unopened bottle or new lot number of commercially prepared acid reagent. If the blank values remain above 99 mmol/L, contact the manufacturer for technical assistance.

- (6) Titrate the calibrator, controls, and patient sample vials according to the manufacturer's recommendation. Record the results, averaging the duplicate titrations.

## 9.5 Result Reporting

- Check that the controls are within the acceptable range. (Refer to Section 9.7.2.)
- Round results off to the nearest whole number, and if possible, have a supervisor review the calculations.
- If the patient result is less than the analytical sensitivity of the chloridometer (also referred to as the limit of quantitation [LoQ], see Section 9.7.4), report it as "<" the LoQ.
- Include the weight or volume of sweat collected. If performing bilateral collection and analysis, report both chloride concentrations; do not average the values. Do not report chloride concentration on inadequate sweat samples.
- Compare the bilateral sweat chloride values. They should agree within 10 mmol/L for values less than or equal to 60 mmol/L, and within 15 mmol/L for values greater than 60 mmol/L.
- The laboratory report should specifically state what measurand (analyte) is measured and apply the appropriate measurand (analyte)-specific reference interval. For example, it is important to apply sweat chloride reference interval to chloride determination and apply distinctly different reference intervals if sweat conductivity is measured.

## 9.6 Procedural Notes and Precautions

- Do not allow any portion of the materials used in the analysis to contact the hands. Use powder-free gloves.
- Clean and maintain the electrodes on the chloridometer according to the manufacturer's recommendations.
- Analyze the calibrators, controls, and patient samples at room temperature.
- The calibration adjustment feature of the chloridometer should be used rarely, if ever. If it is adjusted, then the analytical measuring interval (sometimes called analytical measurement range [AMR]) must be validated before patient testing. The instrument should be in the "standard" not "compensate" mode when running patients and controls.
- Determine the acceptable imprecision for duplicate titration readings from the chloridometer for calibrators, controls (see Section 9.7.2), and patient samples.

- In addition to chloride, other halides such as bromide and iodide are also detected using a chloridometer. Therefore, if the sweat sample contains other halides in addition to chloride, they will be detected and can falsely elevate the sweat chloride result.

## 9.7 Method Validation

The following procedures are suggested for method validation (confirm with local or national regulatory organizations if other validation parameters are needed):

- Trueness (accuracy)
- Precision
- Measuring interval
- Reference interval

Because use of patient sweat samples may not be practical for validation studies owing to limited sample volume, suitable materials with known chloride concentrations, including commercially available controls or chloride calibrators, can be used as surrogate specimens. Test sample volumes should correspond to patient testing volumes. For more details on performing method validation, refer to the CLSI Evaluation Protocols series (see the Related CLSI Reference Materials page).

### 9.7.1 Trueness (Accuracy)

Trueness (accuracy) can be demonstrated by assaying matrix-appropriate samples of known concentration such as calibrators prepared gravimetrically from pure sodium chloride.

### 9.7.2 Precision

Samples for precision studies can include controls and calibrators at concentrations appropriate for medical decisions. Examples of concentrations for medical decisions are sweat chloride less than 30 mmol/L, between 30 to 60 mmol/L, and greater than 60 mmol/L. Laboratories should determine the day-to-day, run-to-run, and within-run repeatability along with operator reproducibility by calculating the mean, SD, and coefficient of variation (CV). Greater imprecision occurs at lower chloride concentrations. Target CV at less than 30 mmol/L should be less than 7%, and less than 5% at other medical decision concentrations. CLSI documents EP05<sup>29</sup> and EP15<sup>33</sup> include protocols for evaluating precision.

### 9.7.3 Measuring Interval

Validation of the measuring interval, also called the AMR, should be performed using at least three and preferably five matrix-appropriate samples spanning the range of patient sweat chloride concentration. For example, the coulometric titration method reportable range should be 10 mmol/L to 160 mmol/L. The samples can be calibrators or controls. Validation should be performed at a minimum of every six months, or more frequently when major changes occur to the measurement process. Determination of this range includes assessing the linearity of the method. Although analytical instruments may have an upper measurement range of greater than 160 mmol/L, concentrations above this are not physiologically possible and should not be reported, as contamination or technical error is likely.<sup>47</sup> Refer to CLSI document EP06<sup>30</sup> for information on evaluating the measuring interval.

### 9.7.4 Limit of Quantitation

In addition to evaluating the upper range of reportable results, the laboratory should determine the analytical sensitivity of the sweat chloride method in order to distinguish reportable concentrations from those with excessive uncertainty at very low concentrations. The method should be able to measure accurately sweat chloride at the mean normal concentration (around 10 mmol/L) without any dilution,

concentration, or other pretreatment that is not part of the usual assay process. Sweat concentrations below the LoQ should be reported as “less than,” for example, “< 10 mmol/L.”<sup>2,48</sup>

A variety of ways to assess the LoQ exist. One approach involves selecting chloride target concentrations between 5 mmol/L and 10 mmol/L using chloride calibrators and performing imprecision studies. The LoQ is the concentration at which the imprecision is acceptable (eg, a CV of less than or equal to 20%). For more details on performing LoQ studies, refer to CLSI document EP17.<sup>49</sup>

### 9.7.5 Reference Interval

Reference intervals for sweat chloride have been established by consensus clinical practice guidelines (see Section 13.2). It is important that the trueness of any method be verified to ensure the clinical guidelines can be used properly in an institution.

## 10 Sweat Conductivity

While sweat chloride is the only analyte upon which a diagnosis of CF should be made, laboratory methods are available for determining sweat conductivity.<sup>2</sup> Conductivity is the property of a solution that allows it to conduct a current. The conductivity depends on the concentration and mobility of the ions in the solution and represents a nonselective measurement of ions.<sup>27,50</sup> Sweat conductivity concentration is not equivalent to sweat chloride concentration because of other ions in sweat such as bicarbonate and lactate. Sweat conductivity is usually reported in mmol/L equivalent NaCl, meaning that a pure sodium chloride solution having the same conductivity as the sample would have the molar concentration reported. On average, sweat conductivity is approximately 15 mmol/L higher than sweat chloride.<sup>27</sup> Sweat-conductivity-measuring instruments are available from several manufacturers. However, some of them lack stability and temperature control, introduce air bubbles, and allow for sample evaporation. A conductivity instrument designed specifically for use with the microbore tubing collector that addresses the above concerns was approved by the CFF as a screening method, but only for use outside accredited Cystic Fibrosis Care Centers.<sup>9,10,27</sup> Additionally, point-of-care devices, measuring conductivity, have been developed.<sup>51,52</sup> A sweat conductivity decision level of greater than or equal to 50 mmol/L (equivalent sodium chloride, NaCl) is recommended by the CFF, and the patient should be referred for a quantitative sweat chloride test.<sup>9,10</sup>

## 11 Labeling of Containers

### 11.1 Reagents, Calibrators, and Controls

Label reagents, calibrators, and controls according to contents, date of preparation, date placed in service, conditions for storage, expiration date, and initials of laboratory personnel preparing the solutions.

### 11.2 Weighing Vials and Specimen Containers

Label the weighing vials or specimen containers with sufficient information to prevent misidentification. The labeling system should include the date of the testing and the designation “left” and “right.”

### 11.3 Titration Vials

Label the individual chloridometer titration vials with sufficient information to prevent misidentification of the patient samples, and include “right” and “left” collection site. Individually label the controls, blanks, and calibrator titration vials.

## 12 Quality Control and Quality Assurance

### 12.1 Analytical Quality Control

Sweat control materials are commercially available or can be prepared in-house, and should be processed simultaneously with each patient sample or batch of patient samples and assayed in exactly the same way as the patient sample. The QC materials should be different from the calibrators used to calibrate the analytical instrument. Controls should include concentrations appropriate for medical decisions. Examples of concentrations at medical decisions are sweat chloride less than 30 mmol/L, between 30 to 60 mmol/L, and greater than 60 mmol/L. Controls must fall within established control ranges and should be reviewed periodically with a target imprecision indicated by a CV of less than 7% at concentrations less than 30 mmol/L, and less than 5% at other medical decision concentrations.

With gauze or filter paper collection, 100 µL of the control solution is placed onto another preweighed filter paper or gauze and processed in parallel with the patient samples. The control gauze or filter paper should show a weight increase of 0.1 g. The advantage of this approach is that all aspects of the process, with the exception of the sweat collection, are evaluated (ie, weighings, dilutions, measurements of the chloride concentration, and calculations). Direct analysis of QC material for chloride concentration is insufficient to ensure that all aspects of the test are adequately controlled. However, this is acceptable if an undiluted sample is obtained using a microbore tubing collector.

### 12.2 Quality Assurance

#### 12.2.1 Training and Competency Assessment for Laboratory Personnel

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. The training process and competency assessment must be documented. Misdiagnosis of patients has been attributed to laboratories performing too few tests to maintain proficiency.<sup>53,54</sup>

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 perform, the CFF allows each laboratory to determine the number of tests required for proficiency.<sup>24</sup>

#### 12.2.2 Collection and Analysis in Duplicate (Bilateral Testing)

Bilateral testing (ie, samples collected from two sites) can be a useful mechanism for QA, as a significant discrepancy between the chloride concentration from the two sites may indicate an error in either collection or analysis. The criteria for acceptable agreement between the two sites are not precisely defined, but chloride values usually agree within a few mmol/L of each other with acceptable limits of within 10 mmol/L for values less than or equal to 60 mmol/L, and within 15 mmol/L for values greater than 60 mmol/L. Diagnostically, bilateral test results do not represent two independent sweat chloride test results.

#### 12.2.3 External Quality Assurance

##### 12.2.3.1 Proficiency Testing

Laboratories should participate in some type of external QA such as proficiency testing programs to assess performance.<sup>a</sup> Participants should be aware that the programs primarily assess the analytical portion of the sweat test.

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<sup>a</sup> For example, in the United States, the College of American Pathologists (CAP); in the United Kingdom, the National External Quality Assessment Schemes (NEQAS); and in Australia, the Royal College of Pathologists Australasia Quality Assurance Program (RCPA-QAP).

### 12.2.3.2 Accreditation Agencies

Several accreditation agencies periodically inspect and accredit laboratories using specific criteria that assess both sweat collection and analysis.<sup>40,55</sup>

## 12.3 Continuous Quality Monitoring

The laboratory should collect and periodically review the following:

- QC data
- Total number of patients with insufficient samples (% QNS) and number per individual laboratory personnel performing the collection
- Total number of patients with skin reactions (burns, urticaria) and number per individual laboratory personnel performing the collection
- Performance on external proficiency testing
- Performance on external inspections and accreditation
- Correlation of results on repeat testing on the same patient
- Percentage of positive, intermediate, and negative results
- Correlation of patient results with genotyping and clinical features

## 13 Evaluation of Results

### 13.1 Diagnostic Criteria

Results from the measurement of chloride concentrations in sweat should be interpreted in relation to the patient's age and clinical picture by a physician knowledgeable about CF. The test results should be consistent with the clinical picture; no single laboratory result is sufficient to establish or rule out the diagnosis of CF. The criteria for the diagnosis of CF include the presence of one or more characteristic phenotypic features, or a history of CF in a sibling, or a positive newborn screening test result; and an increased sweat chloride concentration by pilocarpine iontophoresis on two or more occasions, or identification of two CF-causing mutations or demonstration of abnormal nasal epithelial ion transport.<sup>1</sup> A normal sweat chloride value cannot be used as the sole criterion for exclusion of a CF diagnosis.

### 13.2 Recommended Reference Intervals for Sweat Chloride

#### 13.2.1 Infants

Studies of sweat chloride testing in infants have demonstrated that the age at which testing is done is an important consideration when interpreting the sweat chloride value. Based on the available data on sweat chloride test results in healthy and CF-affected infants, the following sweat chloride ( $\text{Cl}^-$ ) reference intervals are recommended for infants up to and including six months:  $\text{Cl}^- \leq 29$  mmol/L is within a normal range;  $\text{Cl}^- = 30$  to 59 mmol/L is intermediate; and  $\text{Cl}^- \geq 60$  mmol/L is indicative of CF. As more data emerge from newborn screening programs, the upper limit of the normal reference interval may need to be lowered. Although sweat chloride values are generally  $\geq 60$  mmol/L in infants with CF, lower values including concentrations  $<30$  mmol/L can occur. Individuals with intermediate results should have sweat

chloride testing repeated and be referred to a CF center with expertise in the diagnosis of CF in infancy. Further evaluation should include an early detailed clinical assessment, more extensive cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation analysis, and repeat sweat chloride testing and follow-up at 6- to 12-month intervals until the diagnosis is clear.<sup>2</sup>

### 13.2.2 Beyond Infancy

Based on the available data on sweat chloride test results beyond infancy, the following sweat chloride reference intervals are recommended for individuals older than 6 months to 18 years of age: CF is very unlikely in individuals with  $\text{Cl}^- \leq 39$  mmol/L;  $\text{Cl}^- = 40$  to 59 mmol/L is intermediate; and  $\text{Cl}^- \geq 60$  mmol/L is indicative of CF. Individuals with intermediate results should have sweat chloride testing repeated and undergo further evaluation, including a detailed clinical assessment and more extensive CFTR gene mutation analysis. Clinical follow-up should occur at 6- to 12-month intervals and repeat sweat chloride testing should be performed periodically, particularly if a change in symptoms occurs, until the diagnosis is clear.<sup>2</sup>

### 13.2.3 Adults

Studies of normal sweat chloride values beyond infancy using current standardized testing procedures remain limited. A study in unaffected adults age 18 to 39 years found moderately elevated sweat chloride concentrations (mean, 31 mmol/L; range 14 to 48 mmol/L).<sup>56</sup>

In a study of sweat chloride values in 282 carefully screened healthy individuals age 5 to 68 years, the median sweat chloride value in each of seven age-based cohorts was well below 60 mmol/L. The upper limit of the 95% confidence interval was in the intermediate range (40 to 59) for those over age 15 years and just under 60 mmol/L for those age 20 to 68 years, none of whom carried the delta F508 mutation. Three healthy subjects age 15 years and older had a sweat chloride value  $\geq 60$  mmol/L.<sup>57</sup>

Although sweat chloride values of 40 to 59 mmol/L (and rarely  $\geq 60$  mmol/L) can occur in individuals without CF, intermediate sweat chloride values (40 to 59 mmol/L) as well as, rarely, sweat chloride values  $< 40$  mmol/L also can occur in individuals with CF. Individuals diagnosed with CF as adults tend to have lower sweat chloride values compared to individuals diagnosed earlier in life. In the US CFF registry, 13.85% of individuals diagnosed with CF as adults had a value  $< 60$  mmol/L.<sup>2,58</sup>

## 13.3 Transient Elevation in Sweat Electrolyte Concentrations

Evidence exists that there can be physiological variability of sweat electrolyte concentrations over time and that there can be elevation of sweat electrolyte values in healthy persons.<sup>59</sup> Transient elevation of sweat electrolyte concentrations has been reported in patients with anorexia nervosa<sup>60</sup> and in association with environmental deprivation and psychosocial failure to thrive.<sup>61</sup>

## 13.4 Intermediate Sweat Chloride Concentrations

Patients who have persistent intermediate sweat chloride concentrations present a difficult diagnostic challenge. In such cases, it is important to carry out CF mutational determination and quantitative assessment of exocrine pancreatic function. Ancillary findings, such as urogenital abnormalities including azoospermia in postpubertal men, isolation of a CF-associated pathogen from the respiratory tract, and radiographic evidence of pansinusitis, may be helpful. Measurement of nasal transepithelial potential differences and response to amiloride and isoproterenol can also be helpful in such cases.<sup>62,63</sup> Approximately 1% of patients with CF exhibit sweat chloride concentrations persistently in the range of 40 to 60 mmol/L in association with chronic pseudomonas bronchitis and preservation of pancreatic function.<sup>64</sup>

## 13.5 Repeat Sweat Chloride Determination

### 13.5.1 Indications

- All positive sweat chloride results should be repeated and/or confirmed with mutation determination; the diagnosis of CF should never be based on a single positive test.
- All intermediate sweat chloride results (chloride concentration 30 to 59 mmol/L for infants and 40 to 59 mmol/L beyond infancy) should be repeated; if results remain in the intermediate range, additional ancillary tests can be helpful.
- The collection and determination of sweat chloride should be repeated in patients with “confirmed” CF who do not follow an expected clinical course. As patients are followed, the clinical, laboratory, and chest radiograph findings should be consistent with the diagnosis of CF. It is especially important to reevaluate those patients in which the initial diagnosis was suggested primarily on the basis of failure to thrive or a positive family history; the clinical features prompting the initial sweat analysis disappear; the course is consistent with asthma without suppurative lung disease; or there is a normal growth pattern without evidence of digital clubbing, pseudomonas colonization, or typical chest radiograph or CT changes.<sup>2</sup>

### 13.5.2 Timing of Repeat Testing

Repeat sweat collection and analysis can be carried out at any time after the initial testing but preferably at a time when the patient is clinically stable, well-hydrated, free of acute intercurrent illness, and not receiving mineralocorticoids. For example, if a patient is sweat tested with bilateral collections in the morning and fails to produce an adequate sample, the patient could be retested once again that same day using a different stimulation and collection site.

## 13.6 Other Diseases Associated With Elevated Sweat Electrolyte Concentrations

A variety of diseases other than CF can be associated with moderately elevated concentrations of sweat electrolytes<sup>65</sup> (refer to Appendix D). However, with few exceptions, these conditions do not represent a problem in differential diagnosis. Many of these case reports are limited to a small number of patients whose sweat electrolyte concentrations returned to normal upon treatment of the disorder.

## 13.7 Sources of Error

Sweat electrolyte values can vary depending on the method of stimulation of sweating, the volume of sweat collected, the sweat secretory rate, and nutritional and hydration status.<sup>66</sup> However, it is rare for these factors to interfere with the diagnostic validity of the results. In general, false-positive results are more frequently seen than false-negative results.<sup>3,67,68</sup> Most errors are caused by use of unreliable methodology, inadequate sweat collection, technical errors, and misinterpretation of the results. Problems are also attributable to inexperienced laboratory personnel and lack of appropriate QA.

Because of methodological problems, confirmation or rejection of the diagnosis of CF should only be based on the results of a quantitative pilocarpine iontophoresis sweat chloride test described in this document. Direct reading *in situ* tests using ISEs or older electrical conductivity measurements, or measurements of osmolality or sodium are not acceptable as diagnostic tests.<sup>2-4,67,68</sup> With direct reading procedures, the amount of sweat collected is not measured, and an adequate sample cannot be ensured. Unheated cup collectors are unsatisfactory because of condensation.<sup>4,8</sup> A conductivity instrument designed specifically for use with the microbore tubing collector was approved by the CFF as a screening method but only for use outside accredited Cystic Fibrosis Care Centers.<sup>9,10</sup>

Technical problems associated with the determination of sweat chloride include

- Failure to obtain an adequate sweat sample
- Skin contamination by salt-containing materials
- Failure to dry adequately the patient's skin before sweat collection
- Evaporation of the sweat sample during collection, transfer, and transport
- Failure to include condensate in the sweat sample when using gauze or filter paper
- Errors in sample weighing, dilution, elution, analysis result computation, and reporting

Errors in interpretation include

- Establishment of a diagnosis of CF on the basis of a single positive test result
- Failure to repeat a test giving intermediate results
- Failure to repeat tests in patients with a negative result but a clinical picture highly suggestive of CF

False-negative sweat electrolyte results have been reported in the presence of edema and hypoproteinemia.<sup>69,70</sup> In such cases, it is mandatory to repeat the test after resolution of the edema.

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**Appendix A. Indications for Sweat Testing**

<b>Pulmonary and Upper Respiratory Tract Indications</b>	<b>Gastrointestinal Indications</b>	<b>Metabolic and Other Indications</b>
Chronic cough	<i>In utero</i> echogenic bowel	Positive family history
Recurrent or chronic pneumonia	Meconium ileus	Positive newborn screening test result
Wheezing*	Meconium plug syndrome	Presence of two CF-causing mutations in fetus
Hyperinflation*	Prolonged neonatal jaundice	Failure to thrive
Tachypnea*	Steatorrhea	Salty taste to skin
Retractions*	Rectal prolapse	Salt crystals on skin
Atelectasis (especially of the right upper lobe)	Mucoid impacted appendix	Salt-depletion syndrome
Bronchiectasis	Late intestinal obstruction	Metabolic alkalosis
Hemoptysis	Recurrent intussusception	Hypoprothrombinemia
Presence of CF-related pathogens	Cirrhosis	Vitamin A deficiency (bulging fontanelle is a key sign)
Nasal polyps	Portal hypertension	Azoospermia
Pansinusitis	Recurrent pancreatitis	Absent <i>vas deferens</i>
Digital clubbing		Scrotal calcification
		Hypoproteinemia
		Edema

\*If persistent or refractory to usual therapy.

## Appendix B. Current Density

Different power sources, both commercial and homemade, are currently in use. The current density, defined as ampere per square meter of electrode surface delivered, is important. (This can be expressed in mA/cm<sup>2</sup>.) An excess current density can cause discomfort and burns.

A current of 4 mA through a 5.1 × 5.1-cm (2.0 × 2.0-in) gauze square rarely causes difficulties. If this current is applied for five minutes, theoretically, 3.4 mg of pilocarpine can be delivered.

$$P = \frac{itMW}{F} = \frac{4 \text{ mA} \cdot 300 \text{ sec} \cdot 271}{96489 \text{ coulombs/mole}} = 3.370 \text{ mg}$$

where

$P$  = mg pilocarpine nitrate delivered to the skin surface,

$i$  = 4.0 mA,

$t$  = 300 seconds,

$F$  = Faraday constant = 96 489 coulombs/mole, and

$MW$  = molecular weight = 271.

Here, the current density is 0.16 mA/cm<sup>2</sup>, the systemic adult dose of pilocarpine is 10 mg, and iontophoresis is probably about 50% efficient. This theoretical dose has not been found to cause any reactions in infants or children.

Electrodes smaller than 5.1 × 5.1 cm (2.0 × 2.0 in) give better skin contact and higher current densities may be used. The microbore tubing collector system uses an electrode of 6.25 cm<sup>2</sup> (2.5 in<sup>2</sup>) and a current of 1.5 mA. The current density is 0.24 mA/cm<sup>2</sup>.

### Appendix C. Concentration of Pilocarpine Nitrate

When an excess of pilocarpine is placed on the positive electrode, the amount of pilocarpine delivered to the skin is controlled by the current used and the time of iontophoresis. An excess of current has obvious disadvantages. As mentioned in Appendix B, a current of 4 mA for five minutes can theoretically deliver 3.37 mg of pilocarpine. A 5.1 × 5.1-cm (2.0 × 2.0-in) gauze square is thoroughly wet by 2 mL or 3 mL of solution. If a 0.2% solution (0.2 g/100 mL) is used, 2 mL will contain

$$\frac{200 \text{ mg}}{100 \text{ mL}} \cdot 2 \text{ mL} = 4 \text{ mg pilocarpine nitrate.}$$

A stronger solution is not harmful; in practice, 0.2% to 0.5% solutions are used.

**Appendix D. Reported Diseases or Conditions Other Than Cystic Fibrosis Associated With an Elevated Sweat Electrolyte Concentration**

Anorexia nervosa	Keratitis, ichthyosis, deafness (KID) syndrome
Atopic dermatitis	Mauriac's syndrome (malnutrition of)
Autonomic dysfunction	Protein-calorie malnutrition
Celiac disease	Pseudohypoaldosteronism
Environmental deprivation	Psychosocial failure to thrive
Familial cholestasis	Systemic lupus erythematosus
Fucosidosis type 1	Triosephosphate isomerase (TPI) deficiency
Glycogen storage disease type 1	Untreated adrenal insufficiency
Hypogammaglobulinemia	Untreated hypothyroidism

**Clinical and Laboratory Standards Institute consensus procedures include an appeals process that is described in detail in Section 8 of the Administrative Procedures. For further information, contact CLSI or visit our website at [www.clsi.org](http://www.clsi.org).**

## Summary of Consensus Comments and Subcommittee Responses

C34-A2: *Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline—Second Edition*

### General

1. About two years ago, a manufacturer brought out a miniaturized version of its sweat collector and coupled it with a built-in conductivity electrode. It was designed for small patients, especially neonates, and incorporates a number of features that can reduce errors. I suggest that CLSI consider addressing this device in a future version of C34.
- **The C34-A3 revision emphasizes sweat chloride, as this is the diagnostic test for CF. The title of the document was updated to reflect this. Sweat conductivity is discussed briefly in Section 10 to include a neonatal conductivity analyzer.**
2. The important issue of storage and transport of samples before analysis is missing from this document. Often, samples have to be transported from the clinic where the sample is taken (and balanced) to a laboratory for Cl<sup>-</sup> analysis. This is the case, for example, in Sweden and is of major concern. For how long and under what conditions can a sample be stored and transported to a laboratory for analysis (UK guidelines suggest three days at 4 °C)? This is not even mentioned in C34-A2.
- **Storage and transport of samples before analysis is now discussed in Section 9.2.**

### Section 8.2, Effects of Patient Age (formerly Section 8.1.1)

3. I am a clinical chemist and I strongly disagree with an affirmation made in C34-A2: “Therefore, it is recommended that sweat testing not be performed on infants younger than 48 hours of age. Thereafter, if an adequate sweat sample is obtained, the results can be used to confirm or exclude the diagnosis of CF.”

I agree with the first sentence, but I strongly disagree with the second one. If you go back to the original reference, you will see in Figure 2 that the mean and standard errors of the means goes from 50.8 on day 1, down to 38.6 on day 2. This is probably the data you used to conclude that you can use data from sample obtained at 2 days of age and greater. However, the data in the figure present the standard deviation of the mean and not the standard deviation of the whole sample. If you use the data in Table 1, which are the same data used to make the first bar in Figure 2, and you calculate the mean and the standard deviation of the population, you will get  $50.9 \pm 26.1$ . This shows that if the distribution of the sample was normal (which is not the case here), 95% of the results would be between 0 and 103.1. Using a cutoff level of 60 mEq/L, this gives 31% false positives. It really does not look that bad when you look at day 1 results in Figure 2.

Now, imagine doing the same thing for day 2 results in Figure 2. Unfortunately, the data are not in the paper and we cannot do the same calculations. However, it is quite easy to see that the percent of false positive, although most probably lower than 31%, will still be unacceptably high (maybe in the 10% range).

Therefore, due to the lack of other data on this subject, you should change the second sentence in the paragraph I cited. Instead of reading: “Therefore, it is recommended that sweat testing not be performed on infants younger than 48 hours of age. Thereafter, if an adequate sweat sample is obtained, the results can be used to confirm or exclude the diagnosis of CF,” it should read something like: “Therefore, it is recommended that sweat testing not be performed on infants younger than 48 hours of age. Thereafter, if an adequate sweat sample is obtained, the results can be used to exclude the diagnosis of CF. In case of a positive result, the child should ideally be retested when 8 days or older in order to decrease as much as possible the rate of false positives.”

Eight days or older is based on results of the same Figure 2, which shows that at the time, the mean is really close to the one observed for a child of 30 to 60 days old. I do agree that this is still an arbitrary cutoff, because we do not know exactly the percent of false positives in each category. However, this would most certainly decrease the number of putative false positives, treated inappropriately and giving enormous inappropriate stress to the parents.

I hope someone interested in my comments will take the time to evaluate my proposition attentively.

- **Sections 8.2 and 13 address this concern and are based on the CFF consensus document (see Reference 2).**

**Clinical and Laboratory Standards Institute consensus procedures include an appeals process that is described in detail in Section 8 of the Administrative Procedures. For further information, contact CLSI or visit our website at [www.clsi.org](http://www.clsi.org).**

## Summary of Delegate Comments and Subcommittee Responses

C34-A3: *Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline—Third Edition*

### General

1. This is the third edition of the North American sweat test guidelines, and I found it pretty conservative and disappointing. There is very little new information in it. It is a consensus rather than an evidence-based guideline, and the committee is heavily weighted by traditional large cystic fibrosis centers. The hot issues in sweat testing at the moment are

\* Its role following newborn screening, with an ever wider spectrum of disease and sweat test results.

\* Use of sweat conductivity as a first line test, given the lack of commercial chloride methods and relatively poor performance of sweat chloride measurement in EQA schemes.

The third edition has just ducked these issues by restricting the guideline to sweat chloride measurements only. Their suggested cutoff for a first line conductivity screen is lower than any other published cutoff, and would result in a large number of referrals for a repeat sweat test using chloride, especially as they do not recommend storing or transporting the original sweat sample for subsequent chloride measurement.

Although newborn screening is referred to, a lot of the text is just lifted from the previous edition, and does not really question whether two abnormal sweat tests are still needed to confirm diagnosis after IRT and mutation testing have already been done.

A new chloride reference range is introduced for infants <6 months, which is needed and may address some of newborn screening false negative results.

There is an incredible amount of detail specified. Do sweat containers really need to be put inside a glove to transport to the laboratory?

- **This revision includes valuable new content such as method validation, a procedure using smaller sample volume for coil collection, and updated reference intervals for infants. The subcommittee's decision to focus on sweat chloride is consistent with practice guidelines for diagnosing CF based on sweat chloride, not sweat conductivity. The degree of procedural detail in the document is necessary for producing quality results. The statements in the section on minimum sample requirements are based on careful deliberation and consensus of the subcommittee. It is not in the scope of this document to cover diagnostic schemes; that information is contained in consensus practice guidelines such as those found in reference 2. CLSI is in the process of forming a subcommittee for a future document on newborn screening for CF that should address some of the commenter's concerns.**

### Section 2, Standard Precautions

2. First paragraph, last sentence – I think it is too strong a statement to say "...all infectious agents...", as there are agents as yet unidentified; "all known infectious agents" would be better.
- **This statement is included in all CLSI documents and is based on recommendations from the Centers for Disease Control and Prevention and CLSI document M29-A3, *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition*. This comment has been forwarded to the subcommittee responsible for the M29 revision.**

Section 4.3.1, Cleanse the Skin

3. We cleanse the skin with reagent grade alcohol only, based on the idea that alcohol may dry the skin, and the reagent grade water serves to hydrate the skin. Omit cleansing with alcohol.
- **The subcommittee believes the alcohol pad is useful to remove any lotions or creams before rinsing and rehydrating with distilled water.**

Section 4.3.3, Limit Iontophoretic Current

4. Given the onset of cystic fibrosis newborn screen sweat testing, and the potential to burn the thin skin of infants, we use a maximum amperage of 2.5 mA for infants less than 3 months old, or if the skin appears translucent and thin. For infants 3 months and younger, set the current limit at 2.5 mA.
- **The text in Section 4.3.3 was revised to read, "...bring the current up slowly to 2.5 to 4 mA." The text in Section 8.4.1 (13) was revised to read, "Set the current to the lowest value and slowly increase the current to 2.5 to 4 mA, and maintain it at this level for five minutes."**

Section 4.3.6, Keep the Electrode Surfaces Clean

5. After induction, we clean the electrodes with alcohol first (disinfection), then sand with emery cloth, and again clean with alcohol to remove any residual debris that could cause burns. Clean with alcohol before sanding with emery cloth, and wipe down with alcohol after sanding.
- **Any additional disinfection such as that described is covered by the statement in Section 4.3.6 "additional electrode and equipment disinfection may be required based on the institution's infectious control practices."**

Section 4.4, Electrical Malfunctions

6. The iontophoresis system should undergo a documented, regular maintenance procedure by medical engineering personnel for voltage leak and current control. Stipulate how often.
- **The following sentence has been added to the third bullet: "Maintenance frequency depends on institutional and other regulatory requirements."**

Section 4.5, Allergic Reactions

7. An epipen is designed for immediate injection and could be used as alternate or sole form of use if an allergic reaction occurs. List epipen as the preferred or alternative item in this section.
- **The epipen has a preselected dosage available and is not appropriate for all patient ages. The epinephrine ampule provides greater flexibility for dosage.**

Section 5.3.3, Other Methods for Sweat Chloride; and Section 9.1, Principle

8. Instrumentation and methodologies are not "approved by regulatory requirements"; sentence needs to be reworded. Change to "...determination should meet appropriate regulatory requirements..." OR "...should be approved by appropriate regulatory agencies (or bodies)..."
- **The second suggested revision was made.**

Section 7.1.5, Gauze and Filter Paper; and Section 8.4.2, Procedural Notes and Precautions

9. Are there any requirements/recommendations for the size and/or type of filter paper that should be used? Add requirements/recommendations if there are any.
- **There are no recommendations beyond what is described.**

Section 7.2.1, On Gauze or Filter Paper

10. Weighing vials should be of sufficient size to hold 8 mL of diluent. This is the amount of diluent used in the chloridometer procedure. Change to 8 mL.
  - **Ten milliliters was selected to allow for sufficient size for mixing 8 mL of diluents and gauze during elution.**
11. We use an additional wrap of parafilm (2 squares by 4 squares) in lieu of waterproof surgical tape or disposable latex-free stretch bandage and paper tape. We then wrap the second layer of parafilm with an ace bandage and tape closed around the patient's arm. Include the option of the following supplies: Two pieces of paraffin wax film cut into squares measuring 3.0 inches, two pieces of paraffin 2 squares by 4 squares, and disposable ace bandage.
  - **The supplies described are sufficient to avoid evaporation.**

Section 7.3, Chloride Determination by Chloridometer

12. Fourth bullet – Recommends a low control of 10 to 30 mmol/L and high control of 100 mmol/L or greater. It should be changed to recommend controls reflecting the medical decision points of 30 (or 40) and 60 mmol/L.
  - **The subcommittee selected the ranges based on the fact that the mean sweat chloride concentration from normal individuals is around 10 mmol/L, and for CF the mean is around 100 mmol/L.**
13. Fourth bullet, first paragraph, last sentence – The last sentence on control stability seems misplaced; move it to after the third paragraph.
  - **The suggested revision was made.**

Sections 8.4, Stimulation and Collection of Sweat on Gauze or Filter Paper or Into Microbore Tubing Collector; and 8.5, Microbore Tubing Collector Stimulation and Collection Procedure

14. There is overlap in the section titles; in comparison with the text, Section 8.4 should be “Stimulation and Collection of Sweat on Gauze or Filter Paper.” Remove duplication in the section title.
  - **The suggested revision was made.**

Section 8.4.1, Gauze or Filter Paper Stimulation and Collection Procedure

15. Step 5. We have done desiccation studies and have found that there is no difference between desiccated specimens and nondesiccated specimens. Therefore, we no longer desiccate. Eliminate desiccation.
  - **Desiccation may be needed in humid conditions. Each laboratory needs to assess the need for desiccator storage.**
16. Step 7. “...check the battery supply...” sounds like making sure there are batteries in the power supply. Is the intent to make sure the batteries have enough remaining charge to last for the test? What remaining charge is needed? Clarify the statement.
  - **The sentence was revised to read: “Before administering the test, ensure the battery charge is sufficient for the testing or follow the manufacturer’s guidelines.”**
17. Step 9. Delete alcohol.
  - **See the response to comment #5.**
18. Step 13. Add the words “Set the current to the lowest value and...” before the first sentence.
  - **The suggested revision was made**

19. Step 19. See comment #11. Additional option: Secure the paraffin wax with an additional sheet of paraffin measuring 2 squares by 4 squares by wrapping this around the limb and creating an airtight seal. Complete by wrapping the limb with a disposable ace bandage and secure with surgical tape.

- **See the response to comment #11.**

#### Sections 8.5.1, Procedural Notes and Precautions; and 8.6, Procedural Notes and Precautions for Stimulation and Collection

20. Both of these sections are labeled “Procedural Notes and Precautions,” and there is overlap in the content. Item 5 in 8.5.1, for example, talks about removing the collector assembly from the patient’s extremity; it seems this would fit better in Section 8.6. Suggest combining these two sections into one, or rename Section 8.5.1 Microbore Tubing Collector Stimulation and Collection Procedure.

- **The section title was revised according to the second suggestion.**

#### Section 9.4, Procedure Using Microbore Tubing Collection

21. Step 3, third sentence –Rearrange the sentence to “Perform the titrations in duplicate when sufficient sample is available...”

- **The suggested revision was made.**

#### Section 9.5, Result Reporting

22. Fifth bullet – Good laboratory practice would be to check that the controls are within acceptable range before doing anything else. Move the fifth bullet to be first in this section.

- **The suggested revision was made.**

#### Section 9.6, Procedural Notes and Precautions

23. Last bullet – There are two possible interpretations of this statement: (1) the bromide or iodide content of a solution can also be quantitated using the chloridometer; or (2) if bromide and/or iodide contaminates the sample, they will also be detected and falsely elevate the chloride result. Clarify the intended meaning.

- **The following sentence was added for clarification: “Therefore, if the sweat sample contains other halides in addition to chloride, they will be detected and can falsely elevate the sweat chloride result.”**

#### Section 11.1, Reagents, Calibrators, and Controls

24. Add the words “...conditions for...” before “storage.” Which personnel initials should be included on the label, the person preparing the solutions or the person who first used it?

- **The suggested revision was made.**

#### Section 11.3, Titration Vials

25. First sentence – Reword the first sentence as follows: “...patient samples and include...”

- **The suggested revision was made.**

#### Section 12.1, Analytical Quality Control

26. The first paragraph states correctly that the controls should be appropriate for medical decisions and mentions 30 and 60 mmol/L as such.

- **Section 12.1 suggests controls of less than 30 mmol/L and greater than 60 mmol/L, which is consistent with the recommendation in Section 7.3 (see the response to comment 12).**
27. First sentence – It would be useful to add information on how to prepare control materials in house. Can the matrix used be water?
- **Preparation of control material is described in Section 7.3.**

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## The Quality Management System Approach

Clinical and Laboratory Standards Institute subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in CLSI document HS01—*A Quality Management System Model for Health Care*. The quality management system approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any health care service’s path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager’s guide. The QSEs are

- |                                    |  |   |  |
|------------------------------------|--|---|--|
| Documents and Records Organization | Equipment Purchasing and Inventory Process Control | Information Management Occurrence Management Assessment—External and Internal | Process Improvement Customer Service Facilities and Safety |
|------------------------------------|--|---|--|

C34-A3 addresses the QSEs indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Documents and Records	Organization	Personnel	Equipment	Purchasing and Inventory	Process Control	Information Management	Occurrence Management	Assessments —External and Internal	Process Improvement	Customer Service	Facilities and Safety
					X C03 EP05 EP06 EP07 EP10 EP15 EP17 M29			EP10	EP07		M29

Adapted from CLSI document HS01—*A Quality Management System Model for Health Care*.

### Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI document GP26—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow, which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

C34-A3 addresses the clinical laboratory path of workflow steps indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Examination ordering	Preexamination			Examination			Postexamination	
	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
	X	X	X	X	X	X		

Adapted from CLSI document HS01—*A Quality Management System Model for Health Care*.

## Related CLSI Reference Materials\*

- C03-A4**      **Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline—Fourth Edition (2006).** This document provides guidelines on water purified for clinical laboratory use; methods for monitoring water quality and testing for specific contaminants; and water system design considerations.
- EP05-A2**      **Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition (2004).** This document provides guidance for designing an experiment to evaluate the precision performance of quantitative measurement methods; recommendations on comparing the resulting precision estimates with manufacturers' precision performance claims and determining when such comparisons are valid; as well as manufacturers' guidelines for establishing claims.
- EP06-A**      **Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (2003).** This document provides guidance for characterizing the linearity of a method during a method evaluation; for checking linearity as part of routine quality assurance; and for determining and stating a manufacturer's claim for linear range.
- EP07-A2**      **Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition (2005).** This document provides background information, guidance, and experimental procedures for investigating, identifying, and characterizing the effects of interfering substances on clinical chemistry test results.
- EP10-A3**      **Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures; Approved Guideline—Third Edition (2006).** This guideline provides experimental design and data analysis for preliminary evaluation of the performance of a measurement procedure or device.
- EP15-A2**      **User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition (2005).** This document describes the demonstration of method precision and trueness for clinical laboratory quantitative methods utilizing a protocol designed to be completed within five working days or less.
- EP17-A**      **Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (2004).** This document provides guidance for determining the lower limit of detection of clinical laboratory methods, for verifying claimed limits, and for the proper use and interpretation of the limits.
- M29-A3**      **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

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\* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

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