CLINICAL AND LABORATORY STANDARDS INSTITUTE®

4th Edition

C34

Sweat Testing: Specimen Collection and Quantitative Chloride Analysis

This guideline describes methods for all aspects of sweat testing, including collection and analysis, results evaluation and reporting, and quality control.

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

C34, 4th ed.
February 2019
Replaces C34-A3

Sweat Testing: Specimen Collection and Quantitative Chloride Analysis

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Abstract

Clinical and Laboratory Standards Institute guideline C34—*Sweat Testing: Specimen Collection and Quantitative Chloride Analysis* describes methods for performing sweat testing for cystic fibrosis diagnosis. Sweat stimulation, collection, and quantitative measurement of sweat chloride are described, along with results evaluation and reporting, quality assurance, and method validation.

Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis*. 4th ed. CLSI guideline C34 (ISBN 978-1-68440-035-5 [Print]; ISBN 978-1-68440-036-2 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2019.

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Suggested Citation

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

Previous Editions:
March 1993, December 1994, June 2000, December 2009

ISBN 978-1-68440-035-5 (Print)
ISBN 978-1-68440-036-2 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic) Volume 39, Number 2

Contents

Foreword

The quantitative measurement of chloride in sweat (commonly called the "sweat test") is used to confirm cystic fibrosis (CF) diagnosis, and sweat chloride levels are used as a biomarker for evaluation of response to mutation-specific drugs used to treat the disorder. With an approximate incidence of 1:3000, CF is the most common life-shortening genetic disease in Caucasians. CF is an autosomal recessive disorder characterized by viscous secretions that affect the exocrine glands, primarily in the lungs and pancreas. Patients with CF have increased sodium, chloride, and potassium concentrations in their sweat.

Two sets of criteria are evaluated to confirm a CF diagnosis. First, a CF diagnosis involves the presence of one of the following[1,2]:

- One or more characteristic phenotypic features
- CF history in a sibling
- A positive newborn screening test result (see CLSI document NBS05[3])
- Prenatal testing performed due to carrier status in both parents, showing two CF-causing mutations

Second, in addition to one of the criteria above, a CF diagnosis involves the presence of one of the following[1]:

- An increased sweat chloride concentration by pilocarpine iontophoresis
  - This must occur on two or more occasions in the absence of a positive newborn screening test or prenatal testing that identifies two CF-causing mutations.
- Identification of two CF-causing mutations
- Demonstration of abnormal nasal epithelial or intestinal mucosal ion transport

Newborn screening has been implemented throughout the United States and in many other regions and countries. It is essential to note that a positive newborn screening test cannot be used to confirm a CF diagnosis, which requires confirmatory sweat chloride testing or demonstration of two CF-causing mutations in a specimen not obtained prenatally or through newborn screening. Furthermore, false-negative results occur with newborn screening, and sweat testing should always be performed when symptoms suggestive of CF occur, regardless of the newborn screening result.

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 varying patient physiology.[2-7] Therefore, comprehensive[2,4-7] guidelines for sweat collection and quantitative chloride measurement in sweat are needed. Performance improvement of such tests can only occur when laboratorians and clinicians are aware of appropriate methods for patient selection, specimen collection, analysis, results evaluation, and quality control. This guideline describes, in detail, the quantitative pilocarpine iontophoresis test for sweat chloride determination, including techniques to minimize the potential for false-positive and false-negative test results. Sweat conductivity screening methods are also mentioned.[2,4-7]

For diagnosis, CF care center accreditors require that sweating be stimulated by pilocarpine iontophoresis and collected in either gauze or filter paper or in coiled tubing collectors, followed by quantitative chloride[8] measurement. At alternative sites, as a screening procedure, conductivity may be measured (see Subchapter 2.4.4). Patients with a sweat conductivity value of 50 millimoles per liter (mmol/L) (equivalent NaCl) or above should have a quantitative sweat chloride measurement.[8]

Overview of Changes

This guideline replaces the previous edition of the approved guideline, C34-A3, published in 2009. Several changes were made in this edition, including:

- Moved procedures for gauze or filter paper collection and analysis to Appendix A because many of these systems are no longer manufactured
- Moved the procedure for sweat chloride analysis using a chloridometer with individual titration vials and the coiled tubing collector to Appendix B because that chloridometer is no longer manufactured
- Expanded discussion of sweat testing in infants following a positive newborn screening test
- Updated reference intervals for sweat chloride concentration

NOTE: The content of this guideline is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

Key Words

Chloridometer, cystic fibrosis, iontophoresis, sweat chloride, sweat testing

Sweat Testing: Specimen Collection and Quantitative Chloride Analysis

Chapter 1: Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- Standard precautions information
- "Note on Terminology" that highlights particular use and/or variation in use of terms and/or definitions
- Terms and definitions used in the guideline
- Abbreviations and acronyms used in the guideline

1.1 Scope

This guideline provides recommendations for sweat stimulation by pilocarpine iontophoresis (specific precautions are noted), sweat collection in filter paper or gauze (see Appendix A) or in a commercial sweat collector using coiled tubing (see Appendix B), and quantitative chloride measurement. The procedure for sweat chloride (chloride ion [Cl^-]) determination using coulometric titration is described. Sweat conductivity screening methods are also mentioned. Sweat chloride test results evaluation, including reference intervals and diagnostic criteria, is described, with an emphasis on sweat chloride testing for newborn cystic fibrosis (CF) screening. Validation studies and QA techniques are discussed, along with analytical and biological error sources.

The intended users of this guideline are laboratory and clinical personnel responsible for collecting sweat specimens, measuring sweat chloride, and evaluating and reporting sweat test results.

Procedures for gauze or filter paper collection and analysis are located in Appendix A because many of these systems are no longer manufactured. Other methods for measuring sweat electrolytes after pilocarpine iontophoresis exist but are not included in this guideline. Some of these methods have significant documented analytical problems, as well as limited diagnostic application.[2,4-7]

1.2 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 known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of bloodborne pathogens. Published guidelines are available that discuss the daily operations of diagnostic medicine in humans and animals while encouraging a culture of safety in the laboratory.[9] For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all known infectious diseases, refer to CLSI document M29.[10]

1.3 Terminology

1.3.1 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 different countries and regions and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. CLSI recognizes its important role in these efforts, and its consensus process focuses on harmonization of terms to facilitate the global application of standards and guidelines.

Table 1 is provided to clarify the intended interpretations of the following terms.

Table 1. Common Terms or Phrases With Intended Interpretations

Term or Phrase	Intended Interpretation
"Needs to" or "must"	Explains an action directly related to fulfilling a regulatory and/or accreditation requirement or is indicative of a necessary step to ensure patient safety or proper fulfillment of a procedure
"Require"	Represents a statement that directly reflects a regulatory, accreditation, performance, product, or organizational requirement or a requirement or specification identified in an approved documentary standard
"Should"	Describes a recommendation provided in laboratory literature, a statement of good laboratory practice, or a suggestion for how to meet a requirement

1.3.2 Definitions

accuracy (of measurement) – closeness of agreement between a measured quantity value and a true quantity value of a measurand.[11]

analyte – component represented in the name of a measurable quantity.[12]

analytical measuring interval – set of values of quantities of the same kind that can be measured by a given measuring instrument or measuring system with specified instrumental measurement uncertainty, under defined conditions[11]; **NOTE:** It is sometimes called the analytical measurement range, 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.

calibrator – measurement standard used in calibration.[11]

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

clinical laboratory reagent water (CLRW) – water that has been purified to achieve specifications related to resistivity (ie, ≥10 MΩ • cm referenced to 25°C), microbial content (ie, total heterotrophic plate count ≤10 colony-forming units/mL), organic impurities (ie, total organic carbon < 500 ng/g [parts per billion]), and particulate count (ie, ≥0.22 µm at, or near, the output stage using a purification system that includes a stage that blocks the passage of particles).

control (control material) – a device, solution, lyophilized preparation, or panel of collected human or animal specimens, or artificially derived materials, 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 should not be used for calibration in the same process in which they are used as controls.

- Trunk (current crossing heart)
  - The electrodes must not be placed so that current flows across the body. The DC iontophoresis is not likely to interfere in any way with the heart rhythm or devices such as pacemakers. Nevertheless, it is a safe practice to avoid unnecessarily subjecting the torso to any electrical current.

- Any area of inflammation (eg, eczema or rash) or with serous or bloody discharge (can cause contamination)[22]

- Tattooed skin (may result in decreased sweat rate and altered electrolyte concentration)[23]

- Areas of dense hair (allows sweat to pool around hair without entering the tubing, making it difficult to establish uniform electrode and collector contact)

With a coiled tubing collector, either the lower arm or thigh may be used as the collection site. On the arm, the negative electrode should be placed halfway between the shoulder and elbow on the inner surface of the upper arm. The positive electrode (subsequent collection site) should be placed on the inner volar surface of the forearm approximately halfway between the elbow and wrist or slightly toward the distal end of the forearm when a suitable smooth location is available (see Figure 2). The collection device should not be placed so close to the wrist that the tendons are palpable, because reasonably thick musculature is needed to properly seal the collector against the skin. When elliptical electrodes are used, the long axis is placed parallel to the axis of the limb. In addition, when the thigh is selected as the collection site, restraining the patient's leg motion to control flexing of the upper leg muscles helps ensure successful collection.

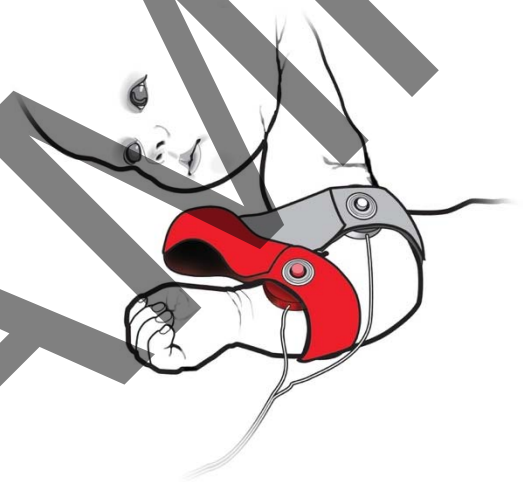

Figure 2. Placement of Electrodes With Coiled Tubing Collector (Created by Mark Irvine, on behalf of ELItechGroup, Inc. Used with permission of ELItechGroup, Inc.)

2.3.2.5 Coiled Tubing Collector Stimulation and Collection Procedure

The procedure for sweat stimulation and collection with a coiled tubing collector is:

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

2. Carefully inspect the pilocarpine-containing gel discs to ensure there are no physical defects.

3. Keep the coiled tubing collector in the wrapper (they are individually wrapped) until it is placed on the patient's skin. To minimize the potential for contamination, the concave collecting surface should not be touched.