CLINICAL AND LABORATORY STANDARDS INSTITUTE®

5th Edition

CLSI C34™

Sweat Testing: Specimen Collection and Quantitative Chloride Analysis

CLSI C34 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.

CLSI C34-Ed5
November 2024
Replaces CLSI C34-Ed4

Sweat Testing: Specimen Collection and Quantitative Chloride Analysis

Uttam Garg, PhD
Ronald Whitely, PhD
Mary Cross, RRT, CPFT, CCRC
Stanley Lo, PhD
Edith T. Zemanick, MD, MSCS
Vicky A. LeGrys, DrA, MT(ASCP)
Dennis Briscoe
Susanna A. McColley, MD

Abstract

Clinical and Laboratory Standards Institute 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, QA, and method validation.

Clinical and Laboratory Standards Institute (CLSI). *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 5th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute, USA, 2024.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Suggested Citation

CLSI. *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 5th ed. CLSI guideline C34. Clinical and Laboratory Standards Institute; 2024.

Previous Editions:
March 1993, December 1994, June 2000, December 2009, February 2019

C34-Ed5

ISBN 978-1-68440-258-8 (Print)

ISBN 978-1-68440-259-5 (Electronic)

ISSN 1558-6502 (Print)

ISSN 2162-2914 (Electronic)

Volume 44, Number 27

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 in Caucasians, CF is diagnosed in many populations worldwide with less disease frequency in other ethnicities.[1] 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[2,3]:

- One or more characteristic phenotypic features
- CF history in a sibling
- A positive newborn screening test result (see CLSI NBS05[4])
- 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[2]:

- An increased sweat chloride concentration by pilocarpine iontophoresis ≥ 60 millimoles per liter (mmol/L)
  - 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 disease-causing mutations in the cystic fibrosis transmembrane conductance regulator gene, one from each parental allele
- Demonstration of abnormal transepithelial nasal potential difference or intestinal current measurement

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 high false-positive or false-negative rates ranging from 7% to 15%, attributable to inaccurate methodology, technical error, and varying patient physiology.[3,5-8] Therefore, comprehensive[3,5-8] guidelines for sweat collection and quantitative chloride measurement in sweat are needed. Performance improvement of such tests can occur only when laboratorians and clinicians are aware of appropriate methods for patient selection, specimen collection, analysis, results evaluation, and QC. CLSI C34 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.[3,5-8]

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[9] 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 mmol/L (equivalent NaCl) or above should have a quantitative sweat chloride measurement.[9]

Overview of Changes

CLSI C34-Ed4 replaces the previous edition of the approved guideline, CLSI 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

CLSI C34 was revised in 2024 under the Limited Revision Process and replaces the 4th edition of the guideline, which was published in 2019. Several changes were made in this edition, including:

- Updating language on sweat stimulation, specimen collection, analysis, and interpretation
- Including conditions that may necessitate consultation with a specialist, such as implanted devices, broken or damaged skin, history of epilepsy or seizures, or pregnancy

NOTE: The content of CLSI C34 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

Chapter 1

Introduction

Sweat Testing: Specimen Collection and Quantitative Chloride Analysis

1 Introduction

1.1 Scope

CLSI C34 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 CLSI C34 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, which are less often performed, 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 CLSI C34. Some of these methods have significant documented analytical problems, as well as limited diagnostic application.[3,5-8]

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.[10] 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 M29.[11]

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 interpretation of common terms.

Appendix A. (Continued)

1a. To prepare a 10-mmol/L chloride control, weigh 0.5844 g of desiccated NaCl and add a sufficient volume of CLRW to bring the volume to 1 L.

1b. To prepare a 50-mmol/L chloride control, weigh 2.922 g of desiccated NaCl and add a sufficient volume of CLRW to bring the volume to 1 L.

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

2. Store controls in tightly stoppered glass bottles at 4°C or as recommended by the manufacturer. The controls are stable for three months or as recommended by the manufacturer. They should be brought to room temperature before use.

3. To assign control values to commercial controls, assay the controls once a day for 20 days and calculate the mean and SDs. For controls that are prepared in house gravimetrically, the weighed-in target value should be used and the SD calculated (see CLSI EP05[5]).

A3 Sweat Stimulation and Collection Procedure

A3.1 Collection Sites

On the arm, the negative electrode should be placed halfway 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 halfway between the elbow and wrist (see Figure A1). To avoid sweat collection problems caused by tendon and wrist flexing during the collection period, the positive electrode should not be too close to the wrist.

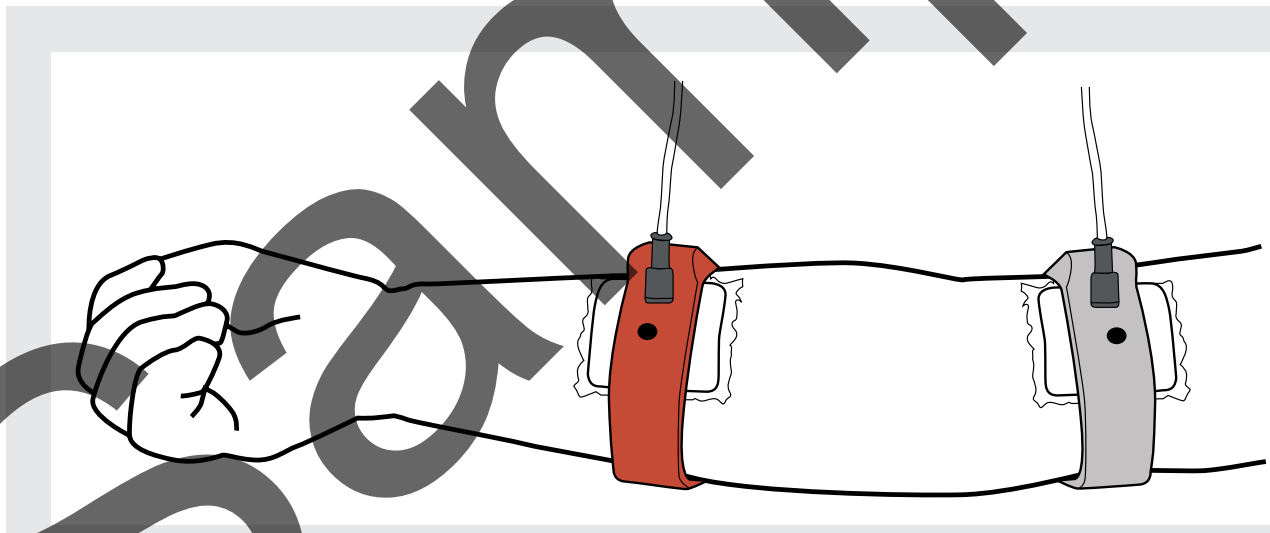

Figure A1. Placement of Electrodes for Gauze or Filter Paper Collection. The pilocarpine is placed on the pad under the positive (red) electrode. The electrolyte solution is placed on the pad under the negative (gray) electrode. (Created by Mark Irvine, on behalf of ELITechGroup, Inc. Used with permission of ELITechGroup, Inc.)

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

PRINT ISBN 978-1-68440-258-8

ELECTRONIC ISBN 978-1-68440-259-5

CLSI C34-Ed5