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

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


The Clinical and Laboratory Standards Institute consensus process, which is the mechanism for moving a document through two or more levels of review by the health care community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of CLSI documents. Current editions are listed in the CLSI catalog and posted on our website at www.clsi.org. If your organization is not a member and would like to become one, and to request a copy of the catalog, contact us at: Telephone: 610.688.0100; Fax: 610.688.0700; E-Mail: customerservice@clsi.org; Website: www.clsi.org.
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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.1,2

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.3-8 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.3-8

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.2 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.9,10

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 (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. \(^3\text{-}^8\) 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, \(^3\text{-}^8\) 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. \(^11\) 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. \(^12\)

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. \(^11\)

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)\(^{13}\); **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)\(^{13}\); **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)\(^{13}\); **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)\(^{13}\).

**analyte** – component represented in the name of a measurable quantity (ISO 17511)\(^{14}\); **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
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
- Personnel
- Equipment
- Purchasing and Inventory
- Information Management
- Process Control
- Occurrence Management
- Assessment—External
- Assessment—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.

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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.

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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.


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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