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C39-A

A Designated Comparison Method for the Measurement of Ionized Calcium in Serum; Approved Standard

This document provides a designated comparison method to standardize the measurement of ionized calcium made by ion-selective electrode (ISE) potentiometry. This system can be used to assign ionized calcium concentrations to a commercially available, serum-based material to improve the traceability and transferability of results for the measurement of ionized calcium in the clinical laboratory.

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A Designated Comparison Method for the Measurement of Ionized Calcium in Serum; Approved Standard

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Abstract

CLSI document C39-A *A Designated Comparison Method for the Measurement of Ionized Calcium in Serum; Approved Standard* provides a candidate designated comparison method to standardize the measurement of ionized calcium made by ion-selective electrode (ISE) potentiometry. This system can be used to assign ionized calcium concentrations to a commercially available, serum-based material to improve the traceability and transferability of results for the measurement of ionized calcium in the clinical laboratory. This standard addresses the principle of the test, the assignment of ionized calcium concentrations to NIST Standard Reference Material 956a, the materials and methods used, and the results and conclusions of an interlaboratory study to assign the ionized calcium concentrations.

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Foreword

The measurement of ionized calcium in whole blood and serum is performed routinely as a service test in many clinical chemistry laboratories. A variety of manufacturers provides modern instrumentation, which allows the rapid measurement of ionized calcium in whole blood and serum with ion-selective membrane electrodes. These instruments, while highly sophisticated, differ in many ways from one manufacturer to another. All of these differences are known to affect the final ionized calcium result.

The purpose of developing this standard is to provide a candidate designated comparison method to standardize the measurement of ionized calcium made by ion-selective electrode (ISE) potentiometry and further, to use this system to assign ionized calcium concentrations to a commercially available, serum-based material to improve the traceability and transferability of results for the measurement of ionized calcium in the clinical laboratory. Development of this standard builds upon both clinical and industrial experience in laboratories around the world and is the result of many years of study of the analytical aspects of iCa^{2+} measurements.

The designated comparison method described in Appendix A of this document may be used to measure the concentration of ionized calcium in serum, not whole blood. The measurement of ionized calcium in whole blood by ISE potentiometry is known to be affected by the presence of erythrocytes. This effect is also present in commercial systems for the measurement of ionized calcium and is variable from one commercial system to another. This document does not address this problem. However, by standardizing the measurement of ionized calcium to a serum based reference material with concentrations assigned by the DCM, the interlaboratory variability for whole blood measurements of ionized calcium would be improved as well.

Key Words

Designated comparison method, ionized calcium, ion-selective electrode, potentiometry, SRM 956a

A Designated Comparison Method for the Measurement of Ionized Calcium in Serum; Approved Standard

1 Introduction

Ionized calcium (iCa^{2+}) has long been recognized as a better indicator of the physiological calcium status in human blood than total calcium.[1-11] The lack of a reference system for iCa^{2+} has been recognized for several years.[12-15] As expected in the absence of a standardization procedure, reference intervals vary from location to location, even for the same type of commercial analyzer, because of differences among instruments, and from one type of commercial analyzer to another. The purpose of developing this standard is to provide a candidate designated comparison method (DCM) to standardize the measurement of ionized calcium made by ion-selective electrode (ISE) potentiometry; and further, to use this method to assign ionized calcium concentrations to a commercially available, serum-based material to improve the traceability and transferability of results for the measurement of ionized calcium in the clinical laboratory. Development of this method was based on both clinical and industrial experience in laboratories around the world, and is the result of many years of study of the analytical aspects of iCa^{2+} measurements.[6,16-19]

2 Scope

This document emphasizes the use of stable, deep-frozen (-50 °C), pooled serum (NIST SRM 956a) with iCa^{2+} values assigned by a designated comparison method (DCM) as the key material which transfers accuracy for the measurement of ionized calcium. The substance concentration of iCa^{2+} in this human serum-based material is determined on the basis of potentiometric comparison to defined standard solutions made from high-purity reference materials. These standards are aqueous solutions whose compositions are established by convention to contain known concentrations of ionized calcium at an ionic strength of 0.160 mol/kg. In general, preparation of these standards follows the recommendations of the Working Group on Selective Electrodes of the International Federation of Clinical Chemistry (IFCC).[15]

The results of a multisite, interlaboratory study using NIST SRM 956a are reported. The objectives of this study were: 1) to show compatibility of the material with various commercial ionized calcium analyzers; and 2) to show usefulness of SRM 956a for providing uniformity to the measurement of ionized calcium in the clinical laboratory.

This document likewise provides specifications for the data acquisition hardware and software components of the ionized calcium DCM. Detailed information is included on the design of the potentiometric ISE and reference half-cells, liquid-liquid junction, and fabrication of tubular, calcium ion-selective membranes. Operating steps for system calibration, sample measurement, and data reduction are also described. Analytical specifications are described in terms of intralaboratory "within-run" and "day-to-day" imprecision to be expected when this technology is mastered.

3 Standard Precautions

Because it is often impossible to know what might be infectious, all human blood specimens are to be treated as infectious and handled according to "standard precautions." Standard precautions are new guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (Guideline for Isolation Precautions in Hospitals, Infection Control and Hospital Epidemiology, CDC, Vol 17;1:53-80.), [MMWR 1987;36(suppl 2S):2S-18S] and (MMWR 1988;37:377-

382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure, refer to NCCLS document M29—*Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*.

4 Principle of the Test

Ionized calcium in solution has been determined by several methods based on different analytical principles: 1) the biological frog heart method[1]; 2) spectrophotometry with calcium ion indicators[2,4,20,21]; and 3) potentiometry with calcium ion-selective electrodes, which responds to changes in thermodynamic activity of calcium ions.[3,22-26] In theory the potentiometric cell responds to ion activity. However, in clinical chemistry, a convention has been adopted which allows standardization and reporting of results from ion-selective electrode potentiometry to be in units of concentration instead of activity. In subsequent sections of this document, concentration will be substituted for activity. See Section A2.1 in Appendix A for further information.

This proposed DCM is based on the third approach— the use of a potentiometric cell made up of two half-cells. The first half-cell is referred to as the "ISE half-cell" and consists of a calcium ion-selective membrane (M), an internal filling solution of fixed calcium ion activity, and a silver-silver chloride (Ag/AgCl) reference electrode (R1). The second half-cell is referred to as the "reference half-cell" and consists of a calomel internal reference electrode (R2) in a solution of saturated potassium chloride. The overall cell configuration may be written as:

R2 / KCl (sat'd) // test solution / M / internal filling solution / R1

reference half-cell ISE half-cell

The connection between the two half-cells is made by a liquid-liquid junction between the test solution and saturated KCl, shown above as "//."

On each side of the calcium ion-selective membrane (M), an electrical potential difference develops across the membrane-solution boundary. The calcium ion-selective membrane for this application is made in a tubular configuration.[17] The outer side of the membrane is in contact with the internal filling solution of constant calcium ion activity and, hence, develops a constant potential. On the inner side of the membrane, the potential varies linearly with the logarithm of the calcium ion activity of the test solution with a response slope of 30.77 millivolts/decade at 37 °C, as governed by the Nernst Equation. Electrodes R1 and R2 are connected to an electrometer and PC-based data acquisition system.

The method is specifically designed for the anaerobic measurement of ionized calcium at 37 °C in serum pools stored frozen at –50 °C or below. In preparation for value assignment measurements, the serum is thawed anaerobically by a set protocol and transferred into the electrochemical cell. The ionized calcium concentration of the sample is calculated by measuring the potential generated by the cell in the presence of the unknown and comparing this value against the potentials generated by a set of known standards.

A measurement made with a potentiometric ion-selective electrode in an undiluted sample represents the concentration of the ion of interest in the water phase of the sample. This measurement bears a variable relationship to concentration of the ion in the entire sample volume as a function of the water content of the sample. Therefore, measurements made with the electrochemical cell described in the DCM should be considered concentrations of ionized calcium in the water phase of the sera. No attempt has been

Related NCCLS Publications*

C29-A **Standardization of Sodium and Potassium Ion-Selective Electrode Systems to the Flame Photometric Reference Method; Approved Standard (1995).** This document provides recommendations on the expression of results of ion-selective electrode measurement of sodium and potassium ion activities in undiluted serum, plasma, or whole blood in clinical practice.

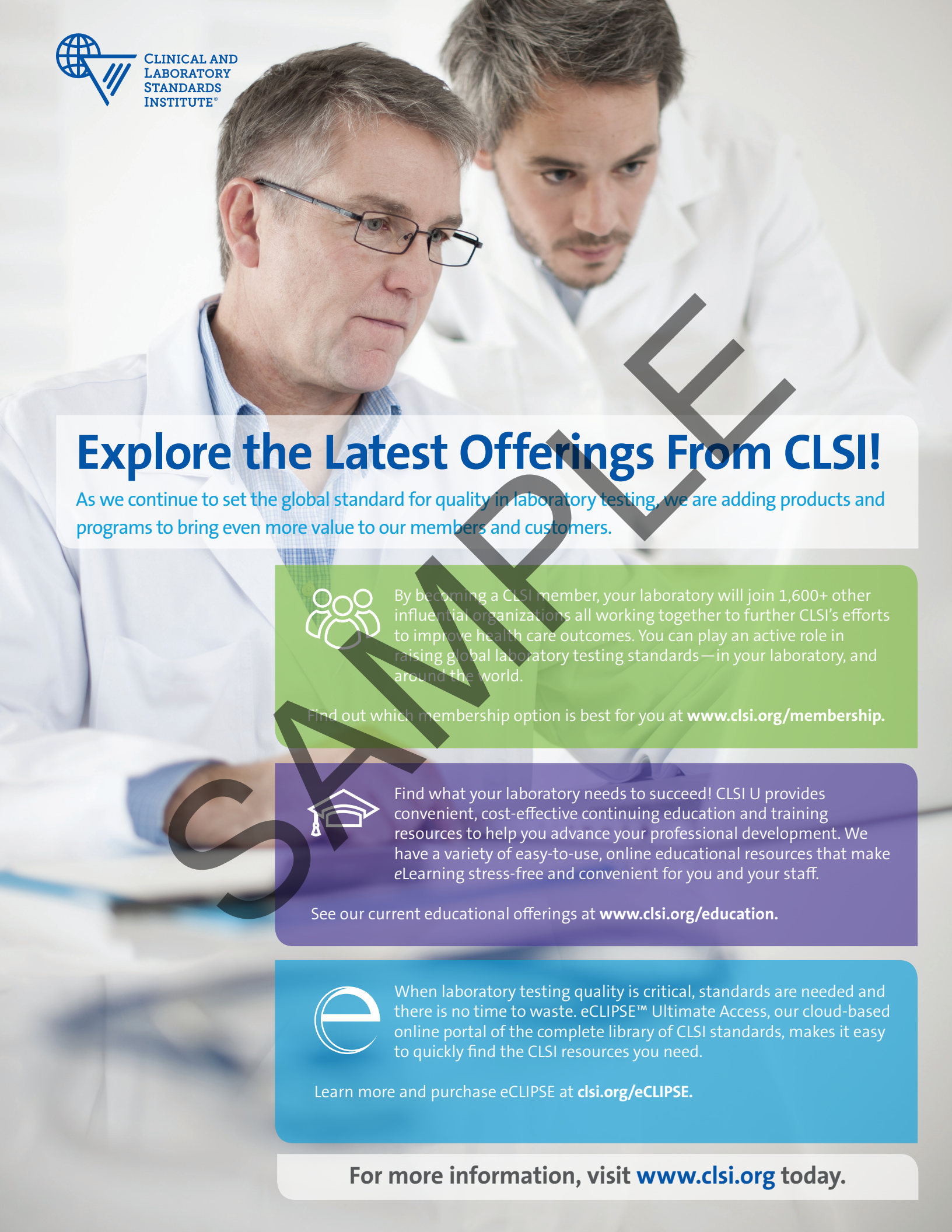
C31-A **Ionized Calcium Determinations: Precollection Variables, Specimen Choice, Collection, and Handling; Approved Guideline (1995).** This document addresses preanalytical considerations — such as patient condition, specimen choice, collection, and handling —that can influence accuracy and clinical utility of ionized calcium measurements.

M29-A **Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline (1997).** This document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

NRSCL8-A **Terminology and Definitions for Use in NCCLS Documents; Approved Standard (1998).** This document provides standard definitions for use in NCCLS standards and guidelines, and for submitting candidate reference methods and materials to the National Reference System for the Clinical Laboratory (NRSCL).

NRSCL13-P **The Reference System for the Clinical Laboratory: Criteria for Development and Credentialing of Methods and Materials for Harmonization of Results; Proposed Guideline (1995)**. This document provides procedures for developing and evaluating definitive methods, reference methods, and reference materials to provide a harmonized clinical measurement system.

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.



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