EP25-A

Evaluation of Stability of *In Vitro* Diagnostic Reagents; Approved Guideline

This document provides guidance for establishing shelf-life and in-use stability claims for *in vitro* diagnostic reagents such as reagent kits, calibrators, and control products.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.
Clinical and Laboratory Standards Institute
Setting the standard for quality in clinical laboratory testing around the world.

The Clinical and Laboratory Standards Institute (CLSI) is a not-for-profit membership organization that brings together the varied perspectives and expertise of the worldwide laboratory community for the advancement of a common cause: to foster excellence in laboratory medicine by developing and implementing clinical laboratory standards and guidelines that help laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability.

Consensus Process

Consensus—the substantial agreement by materially affected, competent, and interested parties—is core to the development of all CLSI documents. It does not always connote unanimous agreement, but does mean that the participants in the development of a consensus document have considered and resolved all relevant objections and accept the resulting agreement.

Commenting on Documents

CLSI documents undergo periodic evaluation and modification to keep pace with advancements in technologies, procedures, methods, and protocols affecting the laboratory or health care.

CLSI’s consensus process depends on experts who volunteer to serve as contributing authors and/or as participants in the reviewing and commenting process. At the end of each comment period, the committee that developed the document is obligated to review all comments, respond in writing to all substantive comments, and revise the draft document as appropriate.

Comments on published CLSI documents are equally essential, and may be submitted by anyone, at any time, on any document. All comments are addressed according to the consensus process by a committee of experts.

Appeals Process

If it is believed that an objection has not been adequately addressed, the process for appeals is documented in the CLSI Administrative Procedures.

All comments and responses submitted on draft and published documents are retained on file at CLSI and are available upon request.

Get Involved—Volunteer!

Do you use CLSI documents in your workplace? Do you see room for improvement? Would you like to get involved in the revision process? Or maybe you see a need to develop a new document for an emerging technology? CLSI wants to hear from you. We are always looking for volunteers. By donating your time and talents to improve the standards that affect your own work, you will play an active role in improving public health across the globe.

For further information on committee participation or to submit comments, contact CLSI.

Clinical and Laboratory Standards Institute
950 West Valley Road, Suite 2500
Wayne, PA 19087 USA
P: 610.688.0100
F: 610.688.0700
www.clsi.org
standard@clsi.org
Evaluation of Stability of *In Vitro* Diagnostic Reagents; Approved Guideline

Volume 29 Number 20

James F. Pierson-Perry  
Sousan S. Altaie, PhD  
Susan J. Danielson, PhD  
Birgitte Lund Jorgensen, PhD  
Bettina Poetsch, PhD  
Rosanne M. Savol, RAC  
Jeffrey E. Vaks, PhD  
Jeffrey Budd, PhD  
Karl De Vore  
Robert Magari, PhD

**Abstract**

Clinical and Laboratory Standards Institute document EP25-A—*Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline* provides guidance and regression-based procedures for establishing stability-related claims of *in vitro* diagnostic (IVD) reagents such as reagent kits, calibrators, control products, and sample diluents. This guideline was written primarily for manufacturers and regulatory agencies, but will also be of interest to clinical laboratories. It provides information on the design, implementation, data analysis, and documentation needs for studies to establish and verify shelf life and in-use life of IVD reagents. Additional topics address assessment of product transport conditions on stability and accelerated stability testing.


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.
Copyright ©2009 Clinical and Laboratory Standards Institute. Except as stated below, any reproduction of content from a CLSI copyrighted standard, guideline, companion product, or other material requires express written consent from CLSI. All rights reserved. Interested parties may send permission requests to permissions@clsi.org.

CLSI hereby grants permission to each individual member or purchaser to make a single reproduction of this publication for use in its laboratory procedure manual at a single site. To request permission to use this publication in any other manner, e-mail permissions@clsi.org.

Suggested Citation


Proposed Guideline
December 2008

Approved Guideline
September 2009

ISBN 1-56238-706-5
ISSN 0273-3099
# Contents

Abstract .................................................................................................................................................... i  
Committee Membership ........................................................................................................................ iii 
Foreword .............................................................................................................................................. vii 
1 Scope .......................................................................................................................................... 1  
2 Standard Precautions .................................................................................................................. 1  
3 Terminology ............................................................................................................................... 1  
   3.1 A Note on Terminology ................................................................................................ 1  
   3.2 Definitions ................................................................................................................... . 2  
   3.3 Abbreviations and Acronyms ....................................................................................... 4  
4 Overview of the Stability Testing Process ................................................................................. 4  
   4.1 Operational Definition of Stability ............................................................................... 5  
   4.2 Types of Stability Studies ............................................................................................. 8  
   4.3 Stability Study Design Options ..................................................................................... 9  
   4.4 The Stability Testing Plan ............................................................................................. 9  
   4.5 Extension to Qualitative Methods ............................................................................... 11  
   4.6 Documentation of Stability Studies ............................................................................ 11  
5 Real-time Stability Study Protocol .......................................................................................... 11  
   5.1 Planning ...................................................................................................................... 11  
   5.2 Experimental ............................................................................................................... 12  
   5.3 Data Analysis .............................................................................................................. 13  
6 Real-time Stability Monitoring (Verification) ......................................................................... 15  
7 Accelerated Stability Testing ................................................................................................... 15  
   7.1 Applications of Accelerated Stability Testing ............................................................ 15  
   7.2 Considerations for Planning Temperature-Based Accelerated Stability Studies .... 16  
   7.3 Analysis of Accelerated Stability Testing Data for Shelf-Life Claims ....................... 17  
References ............................................................................................................................................. 20  
Appendix A. Measurand Drift Analysis Example ................................................................................ 22  
Appendix B. Example of Use of Arrhenius Equation With Accelerated Stability Testing Data to Predict Shelf Life of an *In Vitro* Diagnostic Control Product ................................................................. 24  
Appendix C. Determining the Number of Time Points and Repeats for Stability Studies Based on Linear Regression Analysis .................................................................................................................. 26  
Summary of Comments and Subcommittee Responses ................................................................. 29  
Laboratory Failure Sources and CLSI Evaluation Protocols Documents ..................................... 36  
The Quality Management System Approach ...................................................................................... 38  
Related CLSI Reference Materials .................................................................................................. 39
Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

1 Scope

This guidance document provides information on the establishment and verification of shelf-life and in-use stability claims for quantitative and qualitative in vitro diagnostic (IVD) reagents. It includes background information and typical content to consider when creating a stability testing plan for a particular product, logistics of performing the studies, recommended data analyses, and documentation of stability claims. Additional topics include assessment of product transport conditions on stability claims, stability monitoring (verification), and uses of accelerated stability testing.

The intended users of this guideline are primarily manufacturers of IVD reagents and regulatory agencies. Clinical laboratorians may find this information useful in interpreting commercial product stability claims, as well as for establishing stability attributes of “laboratory-developed test” methods.

This guideline does not address instrument systems, laboratory equipment, software, or patient samples. Stability testing of raw materials or components of reagent kits or consumables is not addressed explicitly. The principles described in this document could, however, be adapted by manufacturers toward that purpose.

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. 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.

3 Terminology

3.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever 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, International Organization of Standardization (ISO), and European Committee for Standardization (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.
3.2 Definitions

**accelerated stability testing** – a stability study designed to increase the rate of chemical or physical degradation of an IVD reagent by using exaggerated environmental conditions (eg, light, temperature, humidity); **NOTE:** Results from such studies may be used to compare the influence of product design/packaging factors or, in some cases, to estimate the expiration date when the product is handled under recommended storage conditions.

**allowable drift** – the maximum change in the quantity value by which product performance is kept within limits specified by the manufacturer.

**Arrhenius equation** – a mathematical function that describes the approximate relationship between the rate constant of a chemical reaction and the reaction temperature and energy of activation.

**bias (of measurement)** – estimate of a systematic measurement error (ISO/IEC Guide 99).7

**calibration** – operation that, under specified conditions, in a first step, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication (ISO/IEC Guide 99)7; **NOTE 1:** Calibration should not be confused with adjustment of a measuring system, often mistakenly called ‘self-calibration,’ nor with verification of calibration (ISO/IEC Guide 99)7; **NOTE 2:** Calibration is the set of operations that establish, under specified conditions, the relationship between reagent system/instrument response and the corresponding concentration/activity values of an analyte.8

**calibration interval** – period of time following a calibration during which an IVD reagent under specified conditions demonstrates apparent change in measurand content within the allowable drift limit and all stability-related criteria are met.

**design input requirements** – the physical and performance requirements of a device that are used as a basis for device design.

**environmental factors** – variables that might affect the performance or efficacy of IVD reagents (eg, temperature, airflow, humidity, light).

**expiration (expiry) date** – upper limit of the time interval during which the performance characteristics of a material stored under specified conditions can be assured; **NOTE:** Expiry dates are assigned to IVD reagents, calibrators, control materials, and other components by the manufacturer based on experimentally determined stability properties (adapted from EN 375:2001, §3.6).9

**first-order kinetics degradation** – a product degradation reaction rate that can be described by a linear differential equation, leading to an exponential relationship between the product concentration and the reaction time.

**in-use stability** – duration of time over which the performance of an IVD reagent within its expiration date remains within specified limits after opening the container system supplied by the manufacturer, and put into use under standard operation conditions (eg, storage on the instrument).

**in vitro diagnostic medical device (IVD medical device)** – a device, whether used alone or in combination, intended by the manufacturer for the *in vitro* examination of specimens derived from the human body to provide information for diagnostic, monitoring, or compatibility purposes. This includes reagents, calibrators, control materials, specimen receptacles, software, and related instruments or apparatus or other articles (GHTF/SG1/N045:2008).10
The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) 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 as follows:

<table>
<thead>
<tr>
<th>Documents and Records</th>
<th>Equipment</th>
<th>Information Management</th>
<th>Process Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organization</td>
<td>Purchasing and Inventory</td>
<td>Occurrence Management</td>
<td>Customer Service</td>
</tr>
<tr>
<td>Personnel</td>
<td>Process Control</td>
<td>Assessments—External and Internal</td>
<td>Facilities and Safety</td>
</tr>
</tbody>
</table>

EP25-A 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.

Adapted from CLSI document HS01—A Quality Management System Model for Health Care.
Related CLSI Reference Materials

**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.


**EP09-A2** Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (2002). This document addresses procedures for determining the bias between two clinical methods, and the design of a method comparison experiment using split patient samples and data analysis.


**EP14-A2** Evaluation of Matrix Effects; Approved Guideline—Second Edition (2005). This document provides guidance for evaluating the bias in analyte measurements that is due to the sample matrix (physiological or artificial) when two measurement procedures are compared.

**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.

**EP18-A** Quality Management for Unit-Use Testing; Approved Guideline (2002). This guideline recommends a quality management system for unit-use devices that will aid in the identification, understanding, and management of sources of error (potential failure modes) and help to ensure correct results. It is targeted for those involved in the supervision of laboratory-testing quality management, and it addresses issues related to specimen collection through reporting of test results.

**EP19-R** A Framework for NCCLS Evaluation Protocols; A Report (2002). This document describes the different types of performance studies that are conducted to evaluate clinical assays.

**EP21-A** Estimation of Total Analytical Error for Clinical Laboratory Methods; Approved Guideline (2003). This document provides manufacturers and end users with a means to estimate total analytical error for an assay. A data collection protocol and an analysis method that can be used to judge the clinical acceptability of new methods using patient specimens are included. These tools can also monitor an assay’s total analytical error by using quality control samples.

* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.
GP10-A  Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristic (ROC) Plots; Approved Guideline (1995). This document provides a protocol for evaluating the accuracy of a test to discriminate between two subclasses of subjects where there is some clinically relevant reason to separate them. In addition to the use of ROC plots, the importance of defining the question, selecting the sample group, and determining the “true” clinical state are emphasized.

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.
Explore the Latest Offerings from CLSI!

As we continue to set the global standard for quality in laboratory testing, we’re adding initiatives to bring even more value to our members and customers.

Power Forward with this Official Interactive Guide
Fundamentals for implementing a quality management system in the clinical laboratory.

Visit the CLSI U Education Center
Where we provide the convenient and cost-effective education resources that laboratories need to put CLSI standards into practice, including webinars, workshops, and more.

Find Membership Opportunities
See the options that make it even easier for your organization to take full advantage of CLSI benefits and our unique membership value.

Shop Our Online Products
Including eCLIPSE Ultimate Access™, CLSI’s cloud-based, online portal that makes it easy to access our standards and guidelines—anytime, anywhere.

For more information, visit www.clsi.org today.