CLINICAL AND LABORATORY STANDARDS INSTITUTE®

2nd Edition

EP25

Evaluation of Stability of *In Vitro* Medical Laboratory Test Reagents

This guideline provides recommendations for establishing and verifying shelf-life and in-use stability claims for *in vitro* diagnostic medical laboratory test reagents such as reagent kits, calibrators, and control products.

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

EP25-Ed2
April 2023
Replaces EP25-A

Evaluation of Stability of *In Vitro* Medical Laboratory Test Reagents

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Abstract

Clinical and Laboratory Standards Institute guideline EP25—*Evaluation of Stability of* In Vitro *Medical Laboratory Test Reagents* provides recommendations and regression-based procedures for establishing and subsequently confirming stability-related claims of *in vitro* medical laboratory 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 medical laboratories and developers of laboratory-developed test methods. It provides information on the design, implementation, data analysis, and documentation needs for studies to establish and confirm shelf life and in-use life of *in vitro* diagnostic products. Additional topics cover the assessment of product transport conditions on stability, use of mean kinetic temperature to reflect product exposure to temperature changes during distribution and storage, and accelerated stability testing.

Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Stability of* In Vitro *Medical Laboratory Test Reagents*. 2nd ed. CLSI guideline EP25 (ISBN 978-1-68440-178-9 [Print], ISBN 978-1-68440-179-6 [Electronic]). Clinical and Laboratory Standards Institute, USA, 2023.

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Suggested Citation

CLSI. *Evaluation of Stability of* In Vitro *Medical Laboratory Test Reagents.* 2nd ed. CLSI guideline EP25. Clinical and Laboratory Standards Institute; 2023.

Previous Edition:
September 2009

EP25-Ed2

ISBN 978-1-68440-178-9 (Print)

ISBN 978-1-68440-179-6 (Electronic)

ISSN 1558-6502 (Print)

ISSN 2162-2914 (Electronic)

Volume 43, Number 8

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Foreword

Stability of an *in vitro* diagnostic (IVD) product reflects its ability to maintain consistent performance characteristics over time. Unlike precision, bias, and other common performance attributes, product stability is rarely assessed directly by end-user testing. As such, there is increased burden on manufacturers and developers of laboratory-developed tests (LDTs) to ensure that stability claims are developed from experimental designs and data analyses that are appropriate for each product's needs and applications.

Products, in the context of this guideline, represent end-user consumable products sold for performing laboratory measurements on patient samples or other samples claimed as appropriate by the manufacturer, such as veterinary or contrived matrixes made from biological materials. Examples of such products are IVD reagent kits or LDTs and their associated calibrators, controls, sample diluents, and system generic reagents. The information also applies to qualitative and semiqualitative or semiquantitative tests.

The content of this guideline is aligned with international standards for stability and internationally recognized guidance documents relative to stability study design and analyses.[1-3] Although these guidance documents were developed for drugs and drug substances, much of their content is directly relevant to IVD reagents.

Overview of Changes

This guideline replaces the previous edition of the approved guideline, EP25-A, published in 2009. Several changes were made in this edition, including:

- Revising the approach to statistical power analysis for planning studies to assume there will be some drift in reagent performance
- Designing a testing plan to demonstrate the drift is within the allowable drift limit
- Eliminating the custom of using the t-test of regression slope results ($P > 0.05$) as a rationale for passing a stability assessment. This practice tends to reward the manufacturer for designing underpowered stability studies.
- Eliminating the requirement for a confidence interval within the acceptance criteria as a basis for stating claims
- Revising the regression analysis approach that now requires point data at the claimed time and beyond the claimed time
- Expanding the practices for transport simulation stability testing
- Adding a discussion about the use of mean kinetic temperature as an integrated measure of temperature changes experienced by a product during distribution and storage
- Expanding the use and practices for accelerated stability testing

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

accelerated stability
allowable drift
equivalence testing
expiry dating
in-use life
isochronous design
mean kinetic temperature
shelf life
stability monitoring
stability plan
transport simulation

Evaluation of Stability of *In Vitro* Medical Laboratory Test Reagents

1 Introduction

1.1 Scope

This guideline provides information for establishing and verifying (confirming) shelf-life and in-use stability claims for quantitative *in vitro* diagnostic (IVD) medical laboratory reagents or products. The information also applies to qualitative IVD products, provided that an underlying continuous response or signal responsible for the qualitative result(s) is available to the investigator. This guideline also includes background information and typical content for creating a stability testing plan, determining the logistics for performing the studies, conducting recommended data analyses, and documenting stability claims for a product. Additional topics include assessment of product distribution conditions on stability claims (transport simulation), verification of stability claims, appropriate uses of accelerated testing, and considerations for testing with difficult samples.

The intended users of this guideline are primarily product manufacturers and regulatory agencies. Medical laboratorians may find this information useful for interpreting and confirming commercial product stability claims (eg, in-use life of QC materials), as well as for establishing stability attributes for laboratory-developed test methods. For this guideline, "products" is understood to encompass reagents, calibrators, controls, diluents, and similar materials that are used as IVD medical devices to conduct a measurement procedure for a measurand of medical interest.

This guideline does not cover instrument systems, laboratory equipment, software, or patient specimens. Stability testing of raw materials or components of reagent kits or consumables is not covered explicitly; however, the principles described in this guideline can be adapted by manufacturers for that purpose.

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.[4] 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.[5]

1.3 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.

As an example, a temperature stress sequence is used in which the on-test product is to be stored at 30°C for 36 hours, followed by storage at 35°C for 12 hours, then finally stored at 45°C for four hours. Temperature measurements are taken every four hours. The E_a for the on-test product is known to be 81.22 kJ/mol. Because the temperature measurements are taken at a constant duration, equation (2) can be used. The calculations per temperature measurement time point are shown in Table 4.

Table 4. Calculation of MKT Intermediate Values

Measurement, i	Temperature, °C	Temperature, K	$e^{\frac{-E_a}{Rt_i}}$
1	30	303.15	1.0130×10^{-14}
2	30	303.15	1.0130×10^{-14}
3	30	303.15	1.0130×10^{-14}
4	30	303.15	1.0130×10^{-14}
5	30	303.15	1.0130×10^{-14}
6	30	303.15	1.0130×10^{-14}
7	30	303.15	1.0130×10^{-14}
8	30	303.15	1.0130×10^{-14}
9	30	303.15	1.0130×10^{-14}
10	35	308.15	1.7088×10^{-14}
11	35	308.15	1.7088×10^{-14}
12	35	308.15	1.7088×10^{-14}
13	45	318.15	4.6283×10^{-14}

Abbreviations: K, kelvin; MKT, mean kinetic temperature.

Symbols: *e*, Euler's number (2.71828); E_a, activation energy; *i*, measurement; *R*, gas constant; t_i, temperature.

Equation (3) is derived from equation (2):

$$MKT = t_k = \frac{\frac{E_a}{R}}{-\ln\left(\frac{e^{\frac{-E_a}{Rt_1}} + e^{\frac{-E_a}{Rt_2}} + \ldots e^{\frac{-E_a}{Rt_N}}}{N}\right)} = 306.6\text{K} = 33.5°\text{C} \quad (3)$$

Using temperature recording devices during product shipments enables MKT to help guide product quality decisions in cases of suspected temperature excursions or unexpected shipment delays. The MKT for the shipment can be calculated from the temperature data. Comparison of that value with the MKT established by the relevant temperature stress sequence used in the transport study indicates whether the material remained within the overall allowable temperature exposure interval.

5 Stability Validation: Analyzing Stability Study Data

5.1 Technical Data Review

The technical data review is an opportunity to communicate results and findings from stability testing with functional area representatives (eg, research and development, quality, regulatory) before finalizing the stability report. A shelf-life stability study for reagent lots is provided as an example. This review is an opportunity for gaining consensus on whether the study objective(s) was met and whether the manufacturer's quality management procedures were followed, as well as for determining which content is appropriate for regulatory submission or registration (when applicable). The technical data review process can include a review of the stability plan, including the desired stability claim, acceptance criteria, and study design. Any observations or deviations that potentially affect the study conclusions should be reviewed. For example, if an instrument malfunctioned during testing, the cause(s) and the resolution(s) should be discussed. When appropriate, it is advantageous for a manufacturer to identify opportunities to improve efficiency and quality of stability testing so that future risk for deviations from the study plan are minimized. This goal can be facilitated by using regression CIs to illustrate the presentation of analyzed data and ensuring initial conclusions are clear and concise. It is helpful to summarize the data analysis procedure in anticipation of questions or clarifications about the stability analysis. Understanding any questions or needed clarifications can be helpful when the stability report is finalized.

Product shelf-life stability is measured by measurand drift (ie, relative change or fixed change in absolute measurand units) for each sample tested and each lot. If appropriate, the measurand drift should be analyzed by the appropriate type of linear regression. In general, measurand drift for each sample and each lot is determined based on these important considerations:

- Regression estimate of drift change, expressed as an absolute difference, percent difference, or ratio value at intended shelf-life claims T_N and T_{N+1}.
- If a model of the average of R replicates vs Time is sufficiently linear, the change is calculated using the linear regression's y-intercept (b_0). If the model is not sufficiently linear, the change is calculated relative to the mean of the replicates calculated at T_0.
- Average of replicates (y-axis) is plotted vs Time (x-axis), and linear regression analysis is applied, if appropriate. Analysis can be conducted with statistical software capable of performing least-squares regression analysis. Using the linear regression analysis equation, measurand drift for each sample and each lot is evaluated at T_N and T_{N+1} along with a 95% one-sided CI. See Appendix D for details on calculating 95% CI limits for measurand drift.
- When totality of the observed measurand drifts for samples in the stability study along with 95% CI is considered, a conclusion about the stability claim at T_N for each lot is obtained by considering Change (%Change) at T_N and T_{N+1} for each sample.
- Although it is not a requirement when claims are reported, it is considered good practice to also evaluate the 95% CI for change in measurand concentration (linear regression model CI) to determine whether it is within (or sufficiently close to) the allowable drift limit while considering that multiple samples were tested for the same lot, and therefore type I error is increased (eg, level of confidence for six samples in the stability study can be as low as 74%, which equals 0.95[6]).
- The claim for T_N should be supported by the stability data from all lots tested. Subchapter 5.2 includes additional information on data analysis including statistical equations, Appendix A includes suggestions for developing sampling plans, and Appendix C includes several stability study examples.