CLINICAL AND LABORATORY STANDARDS INSTITUTE®

January 2011

I/LA28-A2

Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline—Second Edition

This document provides guidelines for the development of validated diagnostic, prognostic, and predictive immunohistochemical assays.

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 medical 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 medical 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 managed according to the consensus process by a committee of experts.

Appeals Process

When it is believed that an objection has not been adequately considered and responded to, the process for appeals, documented in the CLSI Standards Development Policies and Processes, is followed.

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 additional 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: +1.610.688.0100
F: +1.610.688.0700
www.clsi.org
standard@clsi.org

I/LA28-A2
Vol. 31 No. 4
ISBN 1-56238-745-6 Replaces MM04-A
ISSN 0273-3099 Vol. 19 No. 26

Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline—Second Edition

Volume 31 Number 4

Stephen M. Hewitt, MD, PhD
Max Robinowitz, MD
Steven A. Bogen, MD, PhD
Allen M. Gown, MD
Krishan L. Kalra, PhD
Christopher N. Otis, MD
Betsy Spaulding
Clive R. Taylor, MD, DPhil

Abstract

Immunohistochemistry is an analytical technique that applies an antibody reagent to detect and visualize an antigen in cytological and surgical pathology microscopy specimens in the context of histomorphology and cytomorphology. The clinical-pathological interpretation of the presence and patterns of the antibody-antigen reactions is performed in a manner similar to other molecular pathology assays. Immunohistochemistry is used in diagnostic pathology for diagnosis, determination of prognosis, and predictive assays for response to therapy. Accurate and reproducible results require quality assurance of the total test system including the design control of the reagents and the preexamination (preanalytical), examination (analytical), and postexamination (postanalytical) interpretation steps (processes) of the assay to ensure its clinical applicability. This guideline focuses on validation of immunohistochemistry assays on formalin-fixed, paraffin-embedded pathology material. The audience for this guideline includes the assay developer, the reagent supplier, laboratory histotechnologist who performs the assay, and the laboratory director/pathologist who implements and interprets the assay.

Clinical and Laboratory Standards Institute (CLSI). *Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline—Second Edition.* CLSI document I/LA28-A2 (ISBN 1-56238-745-6). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2011.

Suggested Citation

CLSI. *Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline—Second Edition.* CLSI document I/LA28-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

Previous Editions:
July 1997, December 1999

Reaffirmed:
September 2016

ISBN 1-56238-745-6
ISSN 0273-3099

Contents

Contents (Continued)

Contents (Continued)

Foreword

This document is a revision of the previous CLSI document MM04. The goal for the revision is to maintain the consensus recommendations of MM04 while incorporating new information that has accrued since the 1999 publication. The emphasis of this guideline is the application of immunohistochemistry (IHC) and immunocytochemistry (ICC) for the study of human tumor specimens. Other uses, such as identification of microorganisms in specimens, are not addressed.

IHC is just one of the methods intended to assess the diagnosis, prognosis, and prediction of therapy for human tumor specimens. However, IHC and ICC have unique utility through their power to apply immunological methods to localize macromolecular targets within the context of standard histomorphology and cytological morphology. This is particularly useful in tumors that are morphologically anaplastic or are heterogeneous in their cellularity and differentiation.

In the era of personalized or individualized medicine, physicians hope to identify the therapies that are most likely to be effective and avoid therapies that are likely to have unwanted side effects. In order to achieve personalized medicine (ie, individualized medicine), there is a global effort by pharmaceutical and medical device researchers and developers to apply fit-for-purpose method development in the successful identification of biomarkers. There is also an effort to ensure these biomarkers are accurate and reliable for the calculation of the prognosis of the tumor of individual patients, and for predicting optimal response to individualized therapies.[1]

I/LA28-A2 advances the MM04 recommendations for performance of immunohistochemical assays to promote a better understanding of the requirements, capabilities, and limitations of these diagnostic methods; to improve their intra- and interlaboratory reproducibility; and to improve their positive and negative predictive values in diagnosis of disease.

MM04 was revised as I/LA28-A2 because ICC and IHC are based on immunological detection methods, whereas CLSI molecular methods documents are concerned with nucleic acid-based assays. In addition, I/LA28-A2 is intended as a companion document to CLSI documents I/LA23[2] and I/LA21.[3]

This document contains detailed recommendations about preexamination (preanalytical) specimen handling, processing, and preparation. Tissue specimens, especially formalin-fixed, paraffin-embedded tissue are widely used in a variety of different assays today. The inclusion of these preexamination (preanalytical) factors in this document is based on previously published literature and consensus best practices.

Additionally, this document contains recommendations on design and statistical analysis of experiments for estimating the precision of IHC assay results.

Key Words

Anatomical pathology, antigen retrieval, biorepository, formalin fixation, immunocytochemistry, immunohistochemistry, surgical pathology, validation, verification

Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline—Second Edition

1 Scope

The purpose of this document is to provide guidance to all of the stakeholders involved with design and implementation of immunohistochemistry (IHC) assays. It is intended for use by all clinical and reference laboratories performing immunocytochemical assays on cytological preparations or immunohistochemical assays on surgical pathology specimens, for the manufacturers of commercial reagents and test kits, and for individuals and organizations involved in the development and implementation of laboratory quality assurance (QA) programs for these assays.

This guideline presents information on the total product life cycle of the discovery, design, development, verification, and analytical and clinical validation of IHC and immunocytochemistry (ICC) reagents, kits, and systems. Its emphasis is that accurate and reliable IHC and ICC results require attention to the total test system of the assay.

2 Introduction

In preparing this guideline, the subcommittee considered the following needs of stakeholders in the total life cycle of IHC tests:

- Discovery of antigens and antibodies of biological and clinical interest
- Development of research and investigational IHC assays
- Translation to clinical applications: the intended use and indications for use of the test
- Design, verification, and validation of an assay for clinical use
- QA recommendations for laboratory-developed assays and commercialized assays by manufacturers, and for routine clinical laboratory users of IHC assays
- Proficiency testing and other user issues
- Regulation, harmonization, and standardization

The document emphasizes how to address the unique challenges to optimize immunological methodologies applied to histological preparations and cytology.

This document is divided into three parts: Part 1 is the scientific theory and principles of the design and development of IHC assays; Part 2 is the implementation of IHC tests by pathologists and technologists; and Part 3 is the regulation issues and future developments in IHC assays. Figure 1 shows the participants throughout the total IHC life cycle.

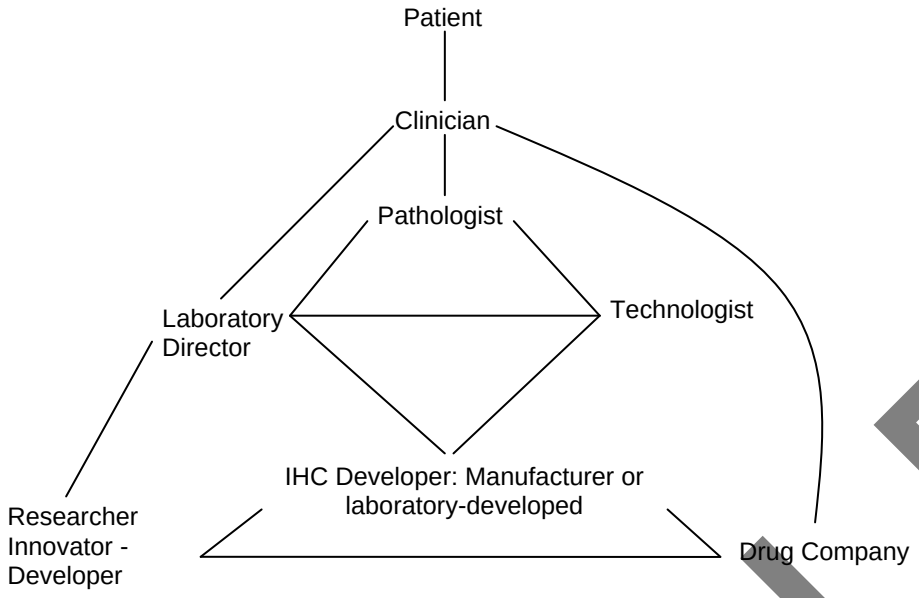

Figure 1. Participants Throughout the Total Immunohistochemistry Life Cycle

Appendix A1 provides statistical points to consider in the evaluation of IHC assays. Appendix B compares and contrasts the characteristics of immunoassays such as enzyme-linked immunosorbent assays (ELISAs) with strengths and limitations of IHC, the combination of immunological methods, and histological and cytological morphology. Appendix C describes the essential features of the concept of the *total test* or total test system as applied to an IHC assay. Total test is the definition of the test that encompasses every step (process) and reagent of the assay, and validation process of the assay as a whole. This is the unifying concept of this guideline. Validation of the assay is based on its clinical utility as a diagnostic, prognostic, or predictive test, and does not require a rigorous molecular biology "truth." Appendix D is a risk-based regulatory checklist for manufacturers of IHC assays to use to check the completeness of their submissions for regulatory clearance or approval for the marketing of new IHC assays in the United States.

For ligand-binding assays, the concept of a total test assay is not unusual. In ELISAs, the antibody pair and detection reagents, as well as assay conditions are fundamental to the assay specification. Unfortunately, in IHC, these features of a "total test" are less appreciated and less applied.

See references from Taylor and Cote[4-6] for the history of the invention and development of IHC.

3 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 blood-borne pathogens. Standard and universal precaution guidelines are available from the US Centers for Disease Control and Prevention.[7] 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.[8]

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 the most current edition of 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	Equipment	Information Management	Process Improvement
Organization	Purchasing and Inventory	Occurrence Management	Customer Service
Personnel	Process Control	Assessments—External and Internal	Facilities and Safety

I/LA28-A2 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 page 138.

Documents and Records	Organization	Personnel	Equipment	Purchasing and Inventory	Process Control	Information Management	Occurrence Management	Assessments—External and Internal	Process Improvement	Customer Service	Facilities and Safety
AST04 I/LA21		AST04 I/LA21	AST04 I/LA21		X AST04 C24 EP05 EP12 EP15 EP19 EP21 GP28 I/LA02 I/LA21 I/LA23 MM06 MM17	 I/LA21		 I/LA21 MM06	 I/LA21	 I/LA23	 I/LA21 M29

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.

I/LA28-A2 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.

Preexamination				**Examination**			**Postexamination**	
Examination ordering	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
			X	X			X	
GP15	AST04 GP15	GP15	GP15	AST04	AST04			GP15
					I/LA02	I/LA02	I/LA02	
MM06	I/LA23 MM06	I/LA23 MM06	I/LA23 MM06	I/LA23 MM06	I/LA23 MM06	I/LA23 MM06	MM06	I/LA23

Adapted from CLSI document HS01—*A Quality Management System Model for Health Care*.

Related CLSI Reference Materials*

AST04-A2 **Glucose Monitoring in Settings Without Laboratory Support; Approved Guideline—Second Edition (2005).** This document contains guidelines for performance of point-of-care (POC) glucose monitoring systems that stress quality control, training, and administrative responsibility.

C24-A3 **Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition (2006).** This guideline provides definitions of analytical intervals, planning of quality control procedures, and guidance for quality control applications.

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.

EP12-A2 **User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition (2008).** This document provides a consistent approach for protocol design and data analysis when evaluating qualitative diagnostic tests. Guidance is provided for both precision and method-comparison studies.

EP15-A2 **User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition (2006).** 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.

EP19-R **A Framework for NCCLS Evaluation Protocols; A Report (2002).** This report 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.

GP15-A3 **Cervicovaginal Cytology Based on the Papanicolaou Technique; Approved Guideline—Third Edition (2008).** This document discusses procedures for cervicovaginal specimen collection, as well as the preparation, fixation, staining, and storage of Papanicolaou-stained cervicovaginal cytology slides.

GP28-A **Microwave Device Use in the Histology Laboratory; Approved Guideline (2005).** This document provides recommendations for reproducing the performance of microwave-accelerated procedures to prepare biological specimens in the histology laboratory.

I/LA02-A2 **Quality Assurance of Laboratory Tests for Autoantibodies to Nuclear Antigens: (1) Indirect Fluorescence Assay for Microscopy and (2) Microtiter Enzyme Immunoassay Methods; Approved Guideline—Second Edition (2006).** This document addresses the criteria for ANA testing by immunofluorescence and by enzyme immunoassay, including test components, quantification of results, and classification criteria.

I/LA18-A2 **Specifications for Immunological Testing for Infectious Diseases; Approved Guideline—Second Edition (2001).** This document addresses specimen collection, handling, and storage, as well as performance criteria for the comparison of immunological test kits and specifications for reference materials.

I/LA21-A2 **Clinical Evaluation of Immunoassays; Approved Guideline—Second Edition (2008).** This document addresses the need for clinical evaluation of new immunoassays and new applications of existing assays, as well as multiple assay formats and their uses. As a guide to designing and executing a clinical evaluation, this document will aid developers of "in-house" assays for institutional use, developers of assays used for monitoring pharmacological effects of new drugs or biologics, and clinical and regulatory personnel responsible for commercializing products.

* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

Related CLSI Reference Materials (Continued)

I/LA23-A **Assessing the Quality of Immunoassay Systems: Radioimmunoassays and Enzyme, Fluorescence, and Luminescence Immunoassays; Approved Guideline (2004).** This guideline addresses components for harmonizing and assessing the quality of immunoassay systems for several commonly used dose-response indicator categories, eg, radioisotopes, enzymes, fluorescence, luminescence, reagents, and experimental components criteria essential to characterizing an immunoassay.

LA01-A2 **Assessing the Quality of Radioimmunoassay Systems—Second Edition; Approved Guideline (1994).** This document contains definitions, procedures, and related information on receptors, radiolabeled analytes, separation reagents, and calibrators for properly assessing radioimmunoassay systems.

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.

MM06-A2 **Quantitative Molecular Methods for Infectious Diseases; Approved Guideline—Second Edition (2010).** This document provides guidance for the development and use of quantitative molecular methods, such as nucleic acid probes and nucleic acid amplification techniques of the target sequences specific to particular microorganisms. It also presents recommendations for quality assurance, proficiency testing, and interpretation of results.

MM17-A **Verification and Validation of Multiplex Nucleic Acid Assays; Approved Guideline (2008).** This guideline provides recommendations for analytical verification and validation of multiplex assays, as well as a review of different types of biological and synthetic reference materials.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Explore the Latest Offerings From CLSI!

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

By becoming a CLSI member, your laboratory will join 1,600+ other influential organizations all working together to further CLSI's efforts to improve health care outcomes. You can play an active role in raising global laboratory testing standards—in your laboratory, and around the world.

Find out which membership option is best for you at **www.clsi.org/membership.**

Find what your laboratory needs to succeed! CLSI U provides convenient, cost-effective continuing education and training resources to help you advance your professional development. We have a variety of easy-to-use, online educational resources that make *e*Learning stress-free and convenient for you and your staff.

See our current educational offerings at **www.clsi.org/education.**

When laboratory testing quality is critical, standards are needed and there is no time to waste. eCLIPSE™ Ultimate Access, our cloud-based online portal of the complete library of CLSI standards, makes it easy to quickly find the CLSI resources you need.

Learn more and purchase eCLIPSE at **clsi.org/eCLIPSE.**

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

CLINICAL AND
LABORATORY
STANDARDS
INSTITUTE®

950 West Valley Road, Suite 2500, Wayne, PA 19087 USA

P: 610.688.0100 **Toll Free (US):** 877.447.1888 **F:** 610.688.0700

E: customerservice@clsi.org **www.clsi.org**

ISBN 1-56238-745-6