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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.

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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.

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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

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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.