

MM07-A2

Fluorescence *In Situ* Hybridization Methods for Clinical Laboratories; Approved Guideline—Second Edition

This document addresses fluorescence *in situ* hybridization methods for medical genetic determinations, identification of chromosomal abnormalities, and gene amplification. Recommendations for probe and assay development, manufacture, qualification, verification, and validation; instrument requirements; quality assurance; and evaluation of results are also included.

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

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Abstract

Clinical and Laboratory Standards Institute document MM07-A2—*Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline—Second Edition* provides information to ensure appropriate and reliable use of the FISH technology. FISH may be used to detect cytogenetic aberrations that are not readily evident by standard cytogenetic banding analyses. FISH technology allows for rapid identification of deletions, duplications, amplifications, and structural abnormalities of specific genes, loci, or chromosomal DNA/RNA sequences. The regions assessed by FISH are typically larger than those studied with PCR, yet smaller than those visualized microscopically with standard cytogenetics. FISH studies have become routine in medical laboratories.

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Contents

Abstract.....	i
Committee Membership.....	iii
Foreword.....	vii
1 Scope.....	1
2 Introduction.....	1
3 Standard Precautions.....	2
4 Terminology.....	2
4.1 A Note on Terminology.....	2
4.2 Definitions.....	2
4.3 Abbreviations and Acronyms.....	4
5 Background.....	5
6 Clinical Applications of FISH.....	5
6.1 Types of Probes.....	6
6.2 Metaphase Applications.....	6
6.3 Interphase Applications.....	7
7 Production of New FISH Probes.....	10
8 Development, Validation, and Verification of FISH Tests.....	10
8.1 What Is the Measurand (Analyte)?.....	11
8.2 What Is the Test?.....	12
8.3 Test Sensitivity and Specificity.....	12
8.4 Introducing a New FISH Test Into the Laboratory.....	15
8.5 Test Reproducibility.....	23
8.6 Controls.....	24
9 Analytical Processes.....	24
9.1 Use of Controls.....	24
9.2 Metaphase Analysis.....	25
9.3 Interphase Analysis.....	25
9.4 Scoring for Chromosome/Target Enumeration.....	27
9.5 Scoring for Fusion and Break-Apart Probe Sets.....	27
9.6 Paraffin-Embedded Tissue Sections.....	27
9.7 Paraffin-Embedded Tissue, Extracted Nuclei.....	28
9.8 Use of FISH for Confirmation of Genomic Microarray Results and Follow-up Family Studies.....	28
10 Reporting.....	28
10.1 Report Contents.....	28
10.2 Disclaimers to Consider With Report.....	29
10.3 Records.....	30
11 Quality Management.....	30

Contents (Continued)

11.1 Personnel.....30

11.2 Documents and Records31

11.3 Quality Control31

11.4 Quality Assurance.....32

12 Equipment Used in FISH Testing33

12.1 Water Baths and Slide Warmers33

12.2 Slides.....33

12.3 Automated FISH Slide Processing.....34

12.4 Thermometers and Pipettes.....34

12.5 Ambient Lighting for Microscopy35

12.6 Microscopes35

12.7 Analytical Automation.....37

References.....38

The Quality Management System Approach40

Related CLSI Reference Materials41

SAMPLE

Foreword

The CLSI Document Development Committee on Fluorescence *In Situ* Hybridization Methods for Medical Genetics was formed to address the need for a guideline on FISH assay development, verification, and clinical validation. This guideline will be useful to clinical laboratories that develop and/or use FISH assays and to agencies that regulate those laboratories. To a lesser extent it may be of value to manufacturers of FISH probes and other reagents used in FISH testing. This guideline expands and revises the previous edition of MM07.

Summary of Major Changes in This Document

- The entire document has been reorganized and updated. Wherever possible, examples have been added to illustrate characteristics of FISH tests.
- FISH technology is being used in settings other than genetic laboratories. Thus, the target audience has been expanded to include all clinical laboratories.
- Although manufacturers of reagents used for FISH testing may find value in knowing the standards applicable to testing laboratories, this guideline no longer includes manufacturing standards.
- Because FISH testing is used heavily in oncology, a greater emphasis has been placed on oncology-related FISH issues in this edition of the guideline.
- Discussion of nonfluorescent detection methods has been added.
- Background information on testing strategies and how FISH testing is used in a clinical setting has been expanded.
- The nature of “measurands (analytes)” detected by FISH testing is discussed in detail and related to other aspects of cytogenetic testing. Also added is a discussion of how FISH testing’s ability to simultaneously detect a potentially large number of analytes impacts test development and test performance.
- Because the measurand (analyte) is sometimes a change in relative position of FISH targets, the sensitivity and specificity of the FISH probe has been distinguished from analytical sensitivity and analytical specificity.
- Statistical methods and a discussion of their limitations for establishing normal cutoff values used to detect mosaicism or the acquired abnormalities associated with neoplasia has been added.
- Discussion of issues pertaining to formalin-fixed, paraffin-embedded samples; samples with selected or enriched cell populations, and samples used for FISH testing in support of microarray analysis has been added.

Note that the methods and QC approaches described in this guideline are based on current clinical applications of FISH testing and that, as new technical methods and clinical applications evolve, other QC methods may be appropriate.

Key Words

Chromosome, cytogenetics, FISH, fluorescence *in situ* hybridization

Fluorescence *In Situ* Hybridization Methods for Clinical Laboratories; Approved Guideline—Second Edition

1 Scope

This document addresses fluorescence *in situ* hybridization methods for medical genetic determinations, identification of chromosomal abnormalities, and gene amplification. Recommendations for probe and assay development, manufacture, qualification, verification, and validation; instrument requirements; QA; and evaluation of results are also included. The guideline is intended to facilitate the reproducible production of FISH assays and the interlaboratory comparison of results and diagnostic interpretations, as well as to ensure accuracy in diagnosis.

This document is intended for use by laboratories that develop tests based on commercially manufactured and laboratory-developed FISH probes. Unlike the previous edition, this revised guideline does not specifically address issues associated with the manufacturing of FISH probes or *in vitro* diagnostic (IVD) devices based on FISH technology. Nevertheless, manufacturers may find value in the principles of FISH testing presented in this guideline and in better understanding how their products will be used by laboratories.

2 Introduction

This guideline primarily addresses “fluorescence” *in situ* hybridization because fluorescence is currently the most widely used method for demonstrating the location of the hybridized probe. Chromogenic *in situ* hybridization (CISH), silver precipitation *in situ* hybridization (SISH), and other nonfluorescent approaches are also in use. Although each of these approaches has its own technical strengths and limitations, the principles of test development and performance are expected to be similar to those described here for fluorescence.

FISH allows geneticists to detect the location of genomic targets (eg, genes, anonymous sequences, and repeat sequences) in a variety of situations. FISH makes use of “probes”: DNA strands with a sequence complementary to a genomic target of interest. Although FISH was originally used primarily for genomic mapping purposes, it has rapidly become an indispensable method for clinical cytogenetic applications. In metaphase cells, FISH can be used to characterize abnormalities detected by conventional chromosome analysis and can also be used to detect abnormalities such as microdeletions and cryptic rearrangements that are not readily detected by conventional chromosome analysis. In interphase cells, FISH can be used to count (enumerate) the number of specific targets in a nucleus and assess changes in the relative position of specific targets. Moreover, the ability to use interphase cells extends the capabilities of cytogenetics by making it possible to include large numbers of cells in FISH analysis and to evaluate tissues with low (or no) mitotic activity.

Presently, venues for probe manufacturing range from contract services to tightly controlled, heavily regulated manufacturing of probes such as those included in US Food and Drug Administration (FDA)–approved test kits. Thus, manufacturing is sufficiently complex to justify one or more guidelines unto itself. Probe manufacturers are encouraged to investigate the regulatory requirements pertinent to the locations in which probes are manufactured and/or sold, and may find value in other CLSI guidelines relating to manufacturing and product stability of reagents intended for laboratory testing (see CLSI document EP25).¹

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. The Centers for Disease Control and Prevention address this topic in published guidelines that address the daily operations of diagnostic medicine in human and animal medicine while encouraging a culture of safety in the laboratory.² 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 M29.³

4 Terminology

4.1 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, 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 and guidelines focuses on harmonization of terms to facilitate the global application of standards and guidelines.

In order to align the usage of terminology in this document with that of ISO, the term *measurand* (a particular quantity subject to measurement) is used in combination with the term *analyte* (component represented in the name of a measurable quantity) when its use relates to a biological fluid/matrix.

The document development committee chose not to replace *clinical sensitivity* and *clinical specificity* with the ISO terms *diagnostic sensitivity* and *diagnostic specificity* owing to user unfamiliarity and for the sake of practicality of the guideline. Outside of the United States, for the most part, the term *clinical* applies to the evaluation of medical products used on or in patients, or when referring to clinical studies of drugs, under much more stringent conditions.

In order to align the usage of terminology in this document with that of ISO and CLSI document QMS01,⁴ the terms *preexamination*, *examination*, and *postexamination* have replaced *preanalytical*, *analytical*, and *postanalytical* when referring to the testing phases within the path of workflow in a laboratory.

4.2 Definitions

analyte – component represented in the name of a measurable quantity (ISO 17511)⁵; **NOTE 1:** In the type of quantity “mass of protein in 24-hour urine,” “protein” is the analyte. In “amount of substance of glucose in plasma,” “glucose” is the analyte. In both cases, the long phrase represents the **measurand** (ISO 17511)⁵; **NOTE 2:** In the type of quantity “catalytic concentration of lactate dehydrogenase isoenzyme 1 in plasma,” “lactate dehydrogenase isoenzyme 1” is the analyte (ISO 18153)⁶; **NOTE 3:** In FISH analysis, the analyte could be viewed as the “genomic target” in “the number of genomic targets per cell” (eg, the number of *BCR* loci detected in a nucleus). It may also be viewed as the “genomic

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 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:

Organization	Personnel	Process Management	Nonconforming Event Management
Customer Focus	Purchasing and Inventory	Documents and Records	Assessments
Facilities and Safety	Equipment	Information Management	Continual Improvement

MM07-A2 addresses the QSE 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.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
QMS01	QMS01	M29 QMS01	QMS01	QMS01	QMS01	X EP05 EP17 EP25 QMS01	QMS01 QMS02	QMS01	QMS01 QMS11	QMS01	QMS01

Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

MM07-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
QMS01	QMS01	QMS01	X QMS01	X QMS01	X QMS01	X QMS01	X QMS01	QMS01

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.
- EP17-A2** **Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition (2012).** This document provides guidance for evaluation and documentation of the detection capability of clinical laboratory measurement procedures (ie, limits of blank, detection, and quantitation), for verification of manufacturers' detection capability claims, and for the proper use and interpretation of different detection capability estimates.
- EP25-A** **Evaluation of Stability of *In Vitro* Diagnostic Reagents; Approved Guideline (2009).** 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.
- 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.
- QMS01-A4** **Quality Management System: A Model for Laboratory Services; Approved Guideline—Fourth Edition (2011).** This document provides a model for medical laboratories that will assist with implementation and maintenance of an effective quality management system.
- QMS02-A6** **Quality Management System: Development and Management of Laboratory Documents; Approved Guideline—Sixth Edition (2013).** This document provides guidance on the processes needed for document management, including creating, controlling, changing, and retiring a laboratory's policy, process, procedure, and form documents in both paper and electronic environments.
- QMS11-A** **Management of Nonconforming Laboratory Events; Approved Guideline (2007).** This guideline provides an outline and the content for developing a program to manage a health care service's nonconforming events that is based on the principles of quality management and patient safety.

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



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