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# 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.

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

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

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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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## 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

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# 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]

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