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Fluorescence *In Situ* Hybridization (FISH) Methods for Medical Genetics; Approved Guideline

This document addresses FISH 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 NCCLS consensus process.

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Fluorescence *In Situ* Hybridization (FISH) Methods for Medical Genetics; Approved Guideline

Russel K. Enns, Ph.D., Chairholder
Gordon Dewald, Ph.D., FACMG, Vice-Chairholder
Peter E. Barker, Ph.D.
Debra J. Rasmussen
Michael Watson, Ph.D., FACMG
Geretta Wood
Philip R. Wyatt, M.D., Ph.D.

Abstract

Fluorescence *in situ* hybridization (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 polymerase chain reaction (PCR), yet smaller than those visualized microscopically with standard cytogenetics. FISH studies have become routine in medical genetics laboratories and this NCCLS guideline provides information to ensure appropriate and reliable use of the technology.

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Committee Membership

Area Committee on Molecular Methods

Russel K. Enns, Ph.D.
Chairholder
Cepheid
Sunnyvale, California

Roberta M. Madej, M.S., M.T.
Vice-Chairholder
Roche Molecular Systems, Inc.
Pleasanton, California

Larry E. Bockstahler, Ph.D.
FDA Ctr. for Devices/Rad. Health
Rockville, Maryland

Timothy J. O'Leary, M.D., Ph.D.
Armed Forces Institute of Pathology
Silver Spring, Maryland

Carolyn Sue Richards, Ph.D., FACMG
Oregon Health Sciences University
Portland, Oregon

Advisors

Lee Ann Baxter-Lowe, Ph.D.
University of California, San Francisco
San Francisco, California

Mark Evans, Ph.D.
American Medical Association
Chicago, Illinois

Linda Ivor
Gen-Probe Inc.
San Diego, California

Robert B. Jenkins, M.D., Ph.D.
Mayo Clinic
Rochester, Minnesota

Alan L. Landay, Ph.D.
Rush-Presby.-St. Lukes Medical Center
Chicago, Illinois

Richard S. Schifreen, Ph.D., DABCC
Promega Corporation
Madison, Wisconsin

Janet L. Wood, M.T.(ASCP)
BD Diagnostic Systems
Sparks, Maryland

Subcommittee on Fluorescence *In Situ* Hybridization (FISH) Methods for Medical Genetics

Russel K. Enns, Ph.D.
Chairholder
Cepheid
Sunnyvale, California

Gordon Dewald, Ph.D., FACMG
Vice-Chairholder
Mayo Clinic
Rochester, Minnesota

Peter E. Barker, Ph.D.
National Institute of Standards & Technology
Gaithersburg, Maryland

Debra J. Rasmussen
Visible Genetics
Alpharetta, Georgia

Eric Shulse
Celera Diagnostics
Alameda, California

Michael Watson, Ph.D., FACMG
American College of Medical Genetics
Bethesda, Maryland

Geretta Wood
FDA Ctr. for Devices/Rad. Health
Rockville, Maryland

Philip R. Wyatt, M.D., Ph.D.
North York General Hospital
Toronto, ON, Canada

Advisors

Janet M. Cowan, Ph.D.
New England Medical Center
Boston, Massachusetts

Colleen Jackson-Cook, Ph.D.
Virginia Commonwealth University
Richmond, Virginia

Joan H.M. Knoll, Ph.D., FACMG, FCCMG
Children's Mercy Hospital
Kansas City, Missouri

James F. Leary, Ph.D.
University of Texas Medical Branch
Galveston, Texas

Louise E. Magruder
FDA Ctr. for Devices/Rad. Health
Rockville, Maryland

Walter C. Mahoney, Ph.D.
Roche Diagnostics
Pleasanton, California

Shivanand Patil, Ph.D., FACMG
University of Iowa Hospital & Clinics
Iowa City, Iowa

Peggy Stupca, M.S., CLSp(CG)
Mayo Clinic
Rochester, Minnesota

Kathryn Sudduth, CLSp(CG), CLSup
University of Virginia HSC
Charlottesville, Virginia

Daynna Wolff, Ph.D., FACMG
Medical University of South Carolina
Charleston, South Carolina

Staff

Lois M. Schmidt, D.A.
Staff Liaison
NCCLS
Wayne, Pennsylvania

Donna M. Wilhelm
Editor
NCCLS
Wayne, Pennsylvania

Melissa A. Lewis
Assistant Editor
NCCLS
Wayne, Pennsylvania

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Foreword

The NCCLS Subcommittee on Fluorescence *In Situ* Hybridization (FISH) Methods for Medical Genetics was formed to address the need for a guideline on FISH assay development, manufacture, verification, and clinical validation of assay performance characteristics, as well as the performance and interpretation of FISH assays in diagnostic genetic laboratories. This guideline should be useful to FISH assay developers, manufacturers, diagnostic genetic laboratories, and regulatory agencies. It is anticipated that this guideline will also be useful in implementing other types of assays that are becoming more frequently used in the medical genetics community.

The chosen methods and quality control approaches are not absolute or immutable. They represent formal recommendations presented by the subcommittee for consensus review, and are intended for use by manufacturers, diagnostic laboratories, and regulatory agencies. Such use 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 guideline is written for laboratory directors, surgical pathologists, medical technologists, other laboratory personnel, geneticists, oncologists, manufacturers of instruments and reagents used in these assays, and those involved in the promulgation of regulations under which laboratories and manufacturers must operate.

Key Words

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

A Note on Terminology

NCCLS, 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. NCCLS 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 NCCLS, ISO, and CEN documents; and that legally required use of terms, regional usage, and different consensus timelines are all obstacles to harmonization. Despite these obstacles, NCCLS recognizes that harmonization of terms facilitates the global application of standards and is an area that needs immediate attention. Implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

In keeping with NCCLS's commitment to align terminology with that of ISO, the following terms are used in MM7: The terms *Clinical evaluation*, *Clinical sensitivity*, and *Clinical specificity* have been replaced with the terms *Diagnostic evaluation*, *Diagnostic sensitivity*, and *Diagnostic specificity* because in Europe, for the most part, the term *clinical* is applied to the evaluation of medical products, which are used on or in patients, or when referring to clinical studies of drugs, under much more stringent conditions.

Users of MM7 should understand, however, that the fundamental meanings of the terms are identical, and to facilitate understanding, the terms are defined along with explanatory notes in the guideline's Definitions section.

Fluorescence *In Situ* Hybridization (FISH) Methods for Medical Genetics; Approved Guideline

1 Scope

FISH technology can be used to detect microdeletions that are not visible by standard cytogenetic banding patterns; it allows for the rapid determination of whether specific genes, loci, or regions are present or if deletions, amplifications, or other structural rearrangements have occurred. This unique ability of FISH technology provides strong justification for its diagnostic use in cancer, prenatal, postnatal, and other genetic diseases.

The methods and quality control approaches described in this guideline represent formal recommendations proposed for use by manufacturers, diagnostic laboratories, and regulatory agencies. Such use 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.

The MM7 guideline has been developed to ensure appropriate and reliable use of FISH technology in medical genetics laboratories and to provide useful recommendations for FISH assay developers, manufacturers, diagnostic genetic laboratories, and regulatory agencies. It is anticipated that this guideline will also be useful in implementing other types of assays that are becoming more frequently used in the medical genetics community.

2 Introduction

Fluorescence *in situ* hybridization (FISH) is commonly used in medical genetics laboratories. FISH reagents, assays, fluorescence microscopy, and image analysis techniques combine to provide high resolution and sensitivity. It is a standard of care for many medical genetic determinations, especially for chromosomal abnormalities and gene amplifications. Many of the test procedures that are used today are "clinical laboratory-developed tests," i.e., the active ingredients of deoxyribonucleic acid (DNA) probes are obtained from commercial sources [e.g., analyte–specific reagents (ASR)] or developed independently by the users together with other reagents and materials necessary to perform these assays. Recently, FISH products with specific indications for use were reviewed and cleared/approved for market as *in vitro* diagnostic (IVD) tests by some regulatory bodies [e.g., the U.S. Food and Drug Administration (FDA) in the United States and the Agence francaise de sécurité sanitaire des produits de santé (AFSSAPS) in France] with more cleared/approved FISH products expected in the near future.

FISH is an international diagnostic technology. Local legislative and regulatory standards must always be followed, in addition to those regulations that must be followed to meet international marketing needs.

3 Terminology

3.1 Definitions

Abnormal reference range – The abnormal reference range should be expressed as a range of percentage of cells with an expected abnormal pattern among patients with a known clinical entity.

Accuracy (of measurement) – Closeness of the agreement between the result of a measurement and a true value of the measurand (VIM93)[1]; **NOTE:** See the definition of **Measurand**, below.

