MM1-A
Vol. 20 No. 7

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Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline

This document provides guidance for the use of molecular biological techniques for clinical detection of heritable mutations associated with genetic disease.

A guideline for global application developed through the NCCLS consensus process.

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Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline

Abstract

NCCLS document MM1-A—*Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline*, provides general recommendations for all phases of the operation of a molecular genetics diagnostic laboratory. The recommendations cover nomenclature for human pedigrees and the designation of mutations; laboratory safety; and "front-end" activities, such as intake information, specimen identification and accessioning, and sample preparation. Other topics addressed are molecular analytical techniques, test validation and characterization, quality assurance, results reporting, and selection of referral laboratories. The guideline also includes definitions of selected terms commonly used in the theory and practice of molecular genetics.

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Volume 20 Number 7

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Foreword

Molecular genetics has now become firmly entrenched as the third major subdiscipline of clinical laboratory medical genetics, emerging more recently than the other subspecialities biochemical genetics and cytogenetics. Just as with any diagnostic method or test, in order to fully benefit the patient, it must be developed and practiced under appropriate conditions. Thus, the purpose of this guideline is to define conditions and principles which will optimize the provision of accurate genetic information while minimizing potential harm to the patient or family.

An important aspect of the development of this and all NCCLS documents should be emphasized, and that is the consensus process. Within the context and operation of NCCLS, the term “consensus” means more than agreement. In the context of guideline development, “consensus” is a process by which NCCLS, its members, and interested parties: 1) have the opportunity to review and to comment on any NCCLS publication, and 2) are assured that their comments will be given serious, competent consideration. In producing MM1-A, the intention of the NCCLS Subcommittee on Molecular Genetics was to reach a consensus so an approved guideline can be distributed to laboratories that use molecular diagnostic tests. The subcommittee also intended for the document to be broad in perspective and, thus, an educational resource for medical genetics.

Note that the following trade names are included in this document Polaroid™; GeneTests.™ It is NCCLS policy to avoid using trade names unless the products identified are the only ones available. Trade names may also be used if they make it significantly easier for the reader to understand and follow the consensus document, as long as all relevant trade names are included.

Key Words

Amplification, gene, genetic disease, molecular diagnostic test, mutation detection, nucleic acid, Southern blot

Acronyms/Abbreviations

ARMS	amplification refractory mutation system
ASO	allele-specific oligonucleotide
bDNA	branched DNA
cDNA	complementary DNA
DGGE	denaturing gradient gel electrophoresis
DNA	deoxyribonucleic acid
dATP	deoxyadenine 5' triphosphate
dCTP	deoxycytidine 5' triphosphate
dGTP	deoxyguanidine 5' triphosphate
dNTPs	deoxyribonucleoside triphosphates
dTTP	deoxythymidine 5' triphosphate
dUTP	deoxyuridine 5' triphosphate
ddATP	dideoxyadenine 5' triphosphate
ddCTP	dideoxycytidine 5' triphosphate
ddGTP	dideoxyguanidine 5' triphosphate
ddTTP	dideoxythymidine 5' triphosphate
dsDNA	double-stranded DNA
EDTA	ethylene diaminetetracetic acid
LCR	ligase chain reaction
NASBA	nucleic acid sequence-based amplification
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism

RNA	ribonucleic acid
RT-PCR	reverse transcriptase-polymerase chain reaction
SDA	strand displacement amplification
SDS	sodium dodecyl sulfate
SSCP	single-stranded conformational polymorphism
STR	short tandem repeat
TAE	tris-acetate-EDTA
TAS	transcription-based amplification system
TBE	tris-borate-EDTA
TE	tris-EDTA
UNG	uracil-N-glycosylase

Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline

1 Introduction

1.1 Diagnostic Utility

The identification, mapping, cloning, and sequencing of numerous genes associated with inherited diseases, and the advent of powerful methods for molecular analysis of these genes in clinical specimens, has revolutionized the practice of medical genetics. With these methods it is now possible to diagnose disease in at-risk individuals prior to the onset of symptoms, to screen for asymptomatic carriers of recessive traits, and to perform prenatal diagnosis even for those diseases which are not expressed in utero. In contrast to the other three major areas of clinical molecular biology—molecular microbiology, molecular oncology, and DNA forensics—where DNA-based techniques supplement or supplant more traditional diagnostic methods, molecular genetic techniques are often the only approach available for the applications cited. As such, they offer a powerful new tool for diagnosis, genetic counseling, and prevention of heritable disease.

1.2 Advantages and Disadvantages

DNA-based tests represent the most fundamental and definitive approach to the diagnosis of genetic diseases which are, by definition, due to lesions in an individual's DNA: nucleotide substitutions, deletions, insertions, duplications, expansions, and inversions. When these lesions are inherited from one or both parents, they are germline defects, present from conception and found in every DNA-containing cell of the body.[a] Unlike molecular diagnosis of neoplasia or infectious disease, which requires DNA sampling of the tumor or infection site, molecular genetic diagnosis can be performed on any accessible body tissue. Even if the biochemical defect is only expressed in a particular inaccessible organ (e.g., the liver), diagnosis can be made using peripheral leukocytes, amniocytes, or any other convenient cell. Where prenatal biochemical diagnosis of phenylketonuria and sickle cell anemia formerly would have required such invasive techniques as fetal liver biopsy and fetal blood sampling, respectively, molecular analysis can now be done on amniocentesis or chorionic villus specimens. Moreover, target amplification techniques such as the polymerase chain reaction (PCR) render even the most minute samples adequate substrates for genetic analysis, leading to sample collection techniques even less invasive than simple phlebotomy: mouthwash, buccal scrapings, random urine collection, dried blood spots, and hair bulb analysis are all viable approaches for obtaining sufficient nucleated cells for mutation detection. In the foreseeable future, the ability to detect and analyze scarce fetal cells circulating in the maternal blood may even render amniocentesis and chorionic villus sampling obsolete. Single-cell genetic analysis may also make preimplantation diagnosis and other applications more commonplace.

The tremendous power of molecular genetic diagnosis brings with it unprecedented clinical, ethical and legal dilemmas and responsibilities. Because this discipline examines the patient's own constitutional genetic makeup, rather than the foreign DNA sequences of an invading microorganism or the secondarily altered genetic complement of a malignancy, the discovery of abnormality, especially in asymptomatic individuals, carries potential risks and liabilities. Identified carriers of mutations, even recessive ones, may become anxious, depressed, or socially stigmatized. They may be discriminated against by employers and health insurers. Presymptomatic diagnosis of severe, untreatable, late-onset disorders such as Huntington disease may be emotionally devastating, even resulting in suicide. Prenatal diagnosis of an affected fetus may lead to the difficult decision to terminate the pregnancy, with all the social, ethical, and legal implications that this entails.

[a] The two sources of human cellular DNA, nuclei and mitochondria, are absent from mature erythrocytes.