TECHNICAL SPECIFICATION

ISO/TS 17822-1

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In vitro diagnostic test systems — Qualitative nucleic acid-based *in vitro* examination procedures for detection and identification of microbial pathogens —

Part 1: General requirements, terms and definitions

Systèmes d'essai pour diagnostic in vitro — Modes opératoires d'examen in vitro qualitatifs fondés sur l'acide nucléique pour la détection et l'identification d'agents pathogènes microbiens —

Partie 1: Exigences générales, termes et définitions

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT), see the following URL: Foreword — Supplementary information.

The committee responsible for this document is ISO/TC 212, *Clinical laboratory testing and in vitro diagnostic test systems*.

ISO/TS 17822 consists of the following parts, under the general title *In vitro diagnostic test systems — Qualitative nucleic acid-based in vitro examination procedures for detection and identification of microbial pathogens*:

— *Part 1: General requirements, terms and definitions*

— *Part 2: Quality practice guide for medical laboratories*

Introduction

Nucleic acid-based *in vitro* diagnostic examination procedures are now commonly used in laboratory medicine for the detection and identification of microbial pathogens. These examination procedures have become particularly valuable for the detection of infectious agents that are difficult to grow in culture. For a review of recent advances and current practices associated with *in vitro* diagnostic examination procedures based on nucleic acid-amplification and detection technology ("molecular diagnostics"), see References [38], [35], [36], [37], [39], [41], and [42].

ISO/TS 17822-1 defines concepts and establishes general principles for the design, development, and performance of qualitative nucleic acid-based *in vitro* diagnostic examinations for the detection and identification of microbial pathogens in human specimens.

Traditional PCR examination procedures typically consist of three steps: (1) sample preparation and nucleic acid extraction, (2) nucleic acid amplification, and (3) nucleic acid detection and identification. The analytical technology is continuing to evolve. Recent kinetic approaches ("real-time PCR") incorporate detection in the amplification step, and multiplex PCR includes the entire system in a cassette.

Due to the inherent complexity and unparalleled analytical sensitivity of nucleic acid-based examination procedures, special attention to their design, development, and use is required, including determination of analytical and clinical performance characteristics, documentation of instructions for use, design of medical laboratory facilities, implementation of appropriate quality assurance practices, verification of the performance characteristics by the medical laboratory in conditions of actual use, and risk management.

As with all *in vitro* diagnostic examination procedures, suitability of a nucleic acid-based examination procedure for its intended clinical uses must be demonstrated as part of the development process. Analytical performance characteristics must be determined and validated for the detection and identification of the target pathogen. Clinical performance characteristics must be determined and validated based on clinical evidence, including evaluation of the benefits and risks to patients. Instructions for use must be clearly documented and effective quality assurance procedures must be specified.

Prior to examination of patient specimens, satisfactory implementation of the examination procedure must be verified by the medical laboratory under conditions of actual use. In other words, the successful transfer of the validated examination procedure from the development laboratory or IVD manufacturer to the end-user medical laboratory must be demonstrated by objective evidence. Any modification of the examination procedure after this transfer may require validation that the analytical and/or clinical performance remains suitable for its intended uses, including reassessment of any risks that could be affected by the modification.

In vitro diagnostic test systems — Qualitative nucleic acid-based *in vitro* examination procedures for detection and identification of microbial pathogens —

Part 1: General requirements, terms and definitions

1 Scope

This Technical Specification is intended for

— IVD medical device manufacturers, medical laboratories, and research and development laboratories that develop nucleic acid-based qualitative *in vitro* diagnostic examination procedures for the detection and identification of microbial pathogens in human specimens, and

— medical laboratories that perform nucleic acid-based *in vitro* diagnostic examinations for the detection and identification of microbial pathogens in human specimens.

This part of ISO/TS 17822 does not apply to

— nucleic acid-based examinations that are not intended for *in vitro* diagnostic use, or

— quantitative nucleic acid-based *in vitro* diagnostic examination procedures.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 13485:2003, *Medical devices — Quality management systems — Requirements for regulatory purposes*

ISO 14971:2007, *Medical devices — Application of risk management to medical devices*

ISO 15189:2012, *Medical laboratories — Requirements for quality and competence*

ISO 15190:2003, *Medical laboratories — Requirements for safety*

ISO 18113-1:2009, *In vitro diagnostic medical devices — Information supplied by the manufacturer (labelling) — Part 1: Terms, definitions and general requirements*

ISO 18113-2:2009, *In vitro diagnostic medical devices — Information supplied by the manufacturer (labelling) — Part 2: In vitro diagnostic reagents for professional use*

ISO 18113-3:2009, *In vitro diagnostic medical devices — Information supplied by the manufacturer (labelling) — Part 3: In vitro diagnostic instruments for professional use*

ISO 23640:2011, *In vitro diagnostic medical devices — Evaluation of stability of in vitro diagnostic reagents*

BIPM JCGM 200:2012, *International vocabulary of metrology — Basic and general concepts and associated terms (VIM), 3rd edition*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 13485, ISO 14971, ISO 15189, ISO 18113-1, JCGM 200, and the following apply.

NOTE The terms and definitions given in ISO 18113-1 take precedence over other sources.

3.1
amplification product
amplicon
nucleic acid products created from a target amplification reaction

Note 1 to entry: Amplicons will be double-stranded DNA if created by a PCR reaction and will be primarily single-stranded RNA if created in a nucleic acid sequence-based amplification or transcription-mediated amplification reaction.

3.2
analytical performance
ability of an examination procedure to measure or detect a particular analyte

[SOURCE: GHTF/SG5/N 6:2012, 4.4.1, modified.]

Note 1 to entry: Analytical performance is determined from analytical performance studies used to assess the ability of an *in vitro* diagnostic examination procedure to measure or detect a particular analyte.

Note 2 to entry: Analytical performance characteristics can include analytical sensitivity, detection limit, analytical specificity (interference and cross-reactivity), trueness, precision, and linearity.

3.3
analytical specificity
capability of a measuring system, using a specified measurement procedure, to provide measurement results for one or more measurands which do not depend on each other nor on any other quantity in the system undergoing measurement

[SOURCE: ISO 18113-1:2009, A.3.4]

Note 1 to entry: Lack of analytical specificity is called analytical interference (see ISO 18113-1:2009, A.3.2).

Note 2 to entry: Lack of analytical specificity in immunochemistry measurement procedures can be due to cross-reactivity (see ISO 18113-1:2009, A.3.12).

Note 3 to entry: Specificity of a measurement procedure should not be confused with clinical specificity (see ISO 18113-1:2009, A.3.16).

Note 4 to entry: JCGM 200:2008 uses the term selectivity for this concept instead of specificity.

Note 5 to entry: For qualitative and semiquantitative examination procedures, analytical specificity is determined by the ability to obtain negative results in concordance with negative results obtained by the reference method.

3.4
annealing
process of hybridization of complementary strands of nucleic acid under specific conditions, for example, as in binding of a primer or a probe to the complementary target nucleic acid sequence

[SOURCE: ISO 22174:2005, 3.4.15]

3.5
clinical accuracy
diagnostic accuracy
(laboratory medicine) ability of an examination procedure to differentiate between patients who have a specific condition and those who do not have the condition

[SOURCE: CLSI EP29-A]

Note 1 to entry: Measures of clinical accuracy include clinical sensitivity and clinical specificity.

Note 2 to entry: Clinical accuracy is affected by the prevalence of the target disease or condition. With the same sensitivity and specificity, clinical accuracy of a particular examination procedure increases as the disease prevalence decreases.

3.6
clinical evaluation
(laboratory medicine) assessment and analysis of clinical evidence in order to verify the clinical safety and performance of an *in vitro* diagnostic examination procedure

[SOURCE: Based on GHTF/SG5/N2R8:2007]

3.7
clinical evidence
(laboratory medicine) all the information that supports the scientific validity and performance for a particular intended use

[SOURCE: GHTF/SG5/N 6:2012, 4.2, modified.]

Note 1 to entry: Clinical evidence or data can include results of any clinical investigations or studies of the *in vitro* diagnostic examination procedure, results of relevant studies reported in the scientific literature, and published or unpublished reports of other clinical experience such as adverse events.

Note 2 to entry: Clinical evidence is used to support the labelling of an IVD medical device, including any claims made about the scientific validity and performance of the device or examination procedure

3.8
clinical performance
(laboratory medicine) ability of an *in vitro* diagnostic examination procedure to yield results that are correlated with a particular clinical condition or physiological state in accordance with the target population and intended user

[SOURCE: GHTF/SG5/N 6:2012, 4.4.2, modified]

Note 1 to entry: Although sometimes referred to as diagnostic performance or clinical validity; clinical performance is the harmonized term endorsed by the Global Harmonization Task Force (GHTF) and its successor, the International Medical Device Regulators Forum (IMDRF).

Note 2 to entry: Evaluation of clinical performance often relies on the outcome of other types of clinical examinations to define “true positive or true negative” results.

3.9
clinical sensitivity
diagnostic sensitivity
(laboratory medicine) ability of an *in vitro* diagnostic examination procedure to identify the presence of a target marker associated with a particular disease or condition

[SOURCE: ISO 18113-1:2009, A.3.15]

Note 1 to entry: Also defined as percent positivity in samples where the target marker is known to be present.

Note 2 to entry: Diagnostic sensitivity is expressed as a percentage (number fraction multiplied by 100), calculated as 100 × the number of true positive values (TP) divided by the sum of the number of true positive values (TP) plus the number of false negative values (FN), or 100 × TP/(TP + FN). This calculation is based on a study design where only one sample is taken from each subject.

Note 3 to entry: The target condition is defined by criteria independent of the examination procedure under consideration.

3.10
clinical specificity
diagnostic specificity
(laboratory medicine) ability of an *in vitro* diagnostic examination procedure to recognize the absence of a target marker associated with a particular disease or condition

[SOURCE: ISO 18113-1:2009, A.3.16]

Note 1 to entry: Also defined as percent negativity in samples where the target marker is known to be absent.

Note 2 to entry: Clinical specificity is expressed as a percentage (number fraction multiplied by 100), calculated as 100 × the number of true negative values (TN) divided by the sum of the number of true negative plus the number of false positive (FP) values, or 100 × TN/(TN + FP). This calculation is based on a study design where only one sample is taken from each subject.

Note 3 to entry: The target condition is defined by criteria independent of the examination procedure under consideration.

3.11
clinical utility
(laboratory medicine) usefulness of the results obtained from an *in vitro* diagnostic examination procedure and the value of the information to the patient and/or the broader population

[SOURCE: GHTF/SG5/N 6:2012, 4.7, modified]

Note 1 to entry: Clinical utility supports clinical decisions for patient management, such as effective treatment or preventive strategies.

3.12
complementary DNA
cDNA
single-stranded DNA that is complementary to a given RNA synthesized in the presence of reverse transcriptase to serve as a template for synthesis of DNA copies

3.13
contamination
introduction of an undesirable substance or matter

3.14
cut-off value
(laboratory medicine) quantity value used as a limit to identify samples that indicate the presence or the absence of a specific disease, condition, or measurand

Note 1 to entry: Defines which measurement results are reported as positive and which are reported as negative.

Note 2 to entry: Measurement results near the cut-off value can be inconclusive due to measurement uncertainty.

Note 3 to entry: The selection of the cut-off value determines the clinical specificity and clinical sensitivity of the examination.

[SOURCE: ISO 18113-1:2009, definition A.3.13]

3.15
denaturation
physical and/or (bio) chemical treatment which destroys or modifies the structural, functional, enzymatic, or antigenic properties of the analyte

[SOURCE: ISO 21572:2013, 3.1.6]

Note 1 to entry: Denaturation of DNA results in separation of double-stranded DNA into single-stranded DNA.

3.16
deoxyribonucleoside triphosphate
dNTP
solution containing dATP, dCTP, dGTP, dTTP, and/or dUTP

[SOURCE: ISO 22174:2005, 3.3.7]

3.17
detection limit
limit of detection
measured quantity value, obtained by a given measurement procedure, for which the probability of falsely claiming the absence of a component in a material is β, given a probability α of falsely claiming its presence

[SOURCE: JCGM 200:2008, 4.18, modified — notes deleted.]

Note 1 to entry: The term analytical sensitivity is sometimes used to mean detection limit, but such usage is now discouraged. See ISO 18113-1:2009, A.2.7 and A.2.8 for further information.

Note 2 to entry: In a nucleic acid-based identification examination, the lowest concentration or content of the target organism per defined amount of matrix that can be consistently detected under the experimental conditions specified in the method.

[SOURCE: ISO 22174:2005, 3.1.8]

3.18
deoxyribonucleic acid
DNA
polymer of deoxyribonucleotides occurring in a double-stranded (dsDNA) or single-stranded (ssDNA) form

[SOURCE: ISO 22174:2005, 3.1.2]

3.19
DNA polymerase for PCR
thermostable enzyme which catalyses repeated DNA synthesis

[SOURCE: ISO 22174:2005, 3.4.17]

3.20
DNA sequencing
determining the order of nucleotide bases (adenine, guanine, cytosine, and thymine) in a molecule of DNA

Note 1 to entry: Sequence is generally described from the 5’ end.

3.21
equipment qualification
verification through inspection, testing, and documentation that the correct equipment has been properly installed and performs according to pre-established specifications

3.22
external amplification control
control DNA added to an aliquot of the extracted nucleic acid in a defined amount or copy number serving as a control for amplification in a separate reaction

[SOURCE: ISO 22174:2005, 3.5.3.2]

3.23
forward work flow
unidirectional work flow
(laboratory medicine) principle of material/sample handling applied to ensure that the primary sample and the processed sample (including amplified DNA) remain physically segregated during the examination procedure

[SOURCE: ISO 24276:2006, modified]

3.24
hybridization
specific binding of complementary nucleic acid sequences under suitable reaction conditions

[SOURCE: ISO 22174:2005, 3.6.3]

3.25
identification
process of recognizing the unique attributes that identify a measurand

Note 1 to entry: In a nucleic acid-based identification examination, the process for determining that an isolate belongs to one of the established target nucleic acid sequences or organisms.

3.26
internal amplification control
DNA added to each reaction in a defined amount or copy number which serves as an internal control for amplification

[SOURCE: ISO 22174:2005, 3.5.3.1]

3.27
mastermix
mixture of reagents needed for PCR, except for the target DNA and the controls

[SOURCE: ISO 22174:2005, 3.4.18]

3.28
multiplex PCR
PCR reaction that uses multiple pairs of primers

[SOURCE: ISO 22174:2005, 3.4.11]

3.29
negative extraction control
extraction blank
control carried through all steps of the nucleic acid extraction procedure in the absence of a test sample

[SOURCE: ISO 22174:2005, 3.5.4]

3.30
negative PCR control
reaction performed with nucleic acid-free water without any PCR inhibitors

[SOURCE: ISO 22174:2005, 3.5.6]

3.31
negative process control
target pathogen-free sample of the collected specimen which is run through all stages of the analytical process

[SOURCE: ISO 22174:2005, 3.5.2, modified]

Note 1 to entry: The nucleic-acid based examination process typically includes sample preparation, enrichment, nucleic acid extraction, and target amplification.

3.32
noncomplementarity
nucleotide bases not aligned antiparallel to each other at each position in the sequence between two DNA or RNA sequences

3.33
nuclease
enzyme that cleaves nucleic acids into smaller nucleotide units

3.34
nuclease inhibitor
substance which blocks nuclease activity

3.35
nucleic acid
macromolecule that is the medium for genetic information or acts as an agent in expressing the information

[SOURCE: ISO 22174:2005, 3.1.1]

Note 1 to entry: There are two types of nucleic acid, DNA and RNA.

3.36
nucleic acid extraction
separation of nucleic acid from other biological materials

Note 1 to entry: Generally to perform amplification and analysis of the nucleic acid.

3.37
nucleic acid primer
strand of nucleic acid that serves as a starting point for DNA synthesis when hybridized to a complementary DNA sequence in the presence of DNA polymerase and deoxyribonucleotide triphosphate

3.38
nucleic acid primer extension
enzymatic reaction which leads to the synthesis of a new DNA strand by the addition of single deoxyribonucleotides to the 3'-end of the primer sequence

[SOURCE: ISO 22174:2005, 3.4.16]

3.39
nucleic acid probe
labelled nucleic acid molecule with a defined sequence used to detect target nucleic acid by hybridization

[SOURCE: ISO 22174:2005, 3.6.1]

3.40
nucleic acid purification
process resulting in a more purified DNA and/or RNA

[SOURCE: ISO 22174:2005, 3.2.2, modified]

3.41
polymerase chain reaction
PCR
enzymatic procedure which allows *in vitro* amplification of DNA

[SOURCE: ISO 22174:2005, 3.4.1]

3.42
PCR-quality DNA
DNA template of sufficient length, purity, and quantity for performing PCR

[SOURCE: ISO 24276:2006, 3.2.3]

3.43
positive PCR control
reaction containing the nucleic acid target in a defined amount or copy number

[SOURCE: ISO 22174:2005, 3.5.5]

3.44
positive process control
sample, containing target nucleic acid, which is treated in the same way as the samples being examined

[SOURCE: ISO 22174:2005, 3.5.1, modified]

Note 1 to entry: The nucleic-acid based examination process typically includes sample preparation, enrichment, nucleic acid extraction, and target amplification.

3.45
reverse transcriptase
enzyme that catalyses reverse transcription

[SOURCE: ISO 22174:2005, 3.3.2]

3.46
reverse transcription
synthesis of cDNA from an RNA template using reverse transcriptase with RT-primer in the presence of deoxyribonucleoside triphosphate

[SOURCE: ISO 22174:2005, 3.3.1, modified]

3.47
ribonuclease
enzyme which degrades RNA

[SOURCE: ISO 22174:2005, 3.3.3]

3.48
ribonuclease inhibitor
substance which blocks ribonuclease activity

[SOURCE: ISO 22174:2005, 3.3.4]

3.49
ribonucleic acid
RNA
polymer of ribonucleotides occurring in a double-stranded or single-stranded form

[SOURCE: ISO 22174:2005, 3.1.3]

3.50
reverse transcription polymerase chain reaction
RT-PCR
method consisting of two reactions, a reverse transcription (RT) of RNA to cDNA and a subsequent PCR

[SOURCE: ISO 22174:2005, 3.4.2, modified]

3.51
RT-PCR quality RNA
RNA template of sufficient length, purity and quantity suitable for reverse transcription and PCR

[SOURCE: ISO 22174:2005, 3.2.4]

3.52
reverse transcription primer
RT-primer
primer used in reverse transcription

[SOURCE: ISO 22174:2005, 3.3.5, modified]

3.53
sequence database
(bioinformatics) biological database consisting of nucleic acid sequences, protein sequences, or other polymer sequences and associated annotation

Note 1 to entry: The annotation can relate to organism, species, function, mutations linked to particular diseases, functional or structural features, bibliographic references, etc.

Note 2 to entry: All published genome sequences are available over the internet, as it is a requirement of every scientific journal that any published DNA or RNA or protein sequence must be deposited in a public database.

3.54
stringency
degree of the conditions used in a reaction, affecting the specificity of hybridization, or annealing or washing

[SOURCE: CLSI MM01:2012, 4.2, modified — Note deleted.]

3.55
target DNA
DNA sequence selected for amplification

[SOURCE: ISO 22174:2005, 3.4.13]

3.56
thermal cycler
automatic device which performs defined heating and cooling cycles necessary for PCR

[SOURCE: ISO 22174:2005, 3.4.20]

4 Principles of nucleic acid based *in vitro* diagnostic examinations

4.1 General requirements

4.1.1 Design and development

Design and development of a nucleic acid-based *in vitro* diagnostic examination procedure, including reagents, equipment, software, and instructions for use, shall follow a documented design and development control process.

Design and development activities, including design and development controls, shall be planned and approved according to established procedures.

NOTE 1 A design and development plan can include the design and development stages; the review, verification, validation, clinical evaluation and transfer activities that are appropriate at each design and development stage, the interfaces between different groups involved in design and development; the risk management plan (see 6.1); and the responsibilities and authorities for design and development.

Design and development controls shall include the following:

a) definition of the intended medical use;

b) performance requirements and other design requirements based on the intended uses;

EXAMPLE Detection limit, cut-off values, analytical specificity (including cross-reactivity and interference), precision, carryover, linearity, and where appropriate, calibrator commutability and traceability of results to reference materials or reference measurement procedures.

c) verification that each design requirement has been met;

d) validation that the performance characteristics are suitable for the intended use;

e) control of subsequent changes to the examination procedure;

f) management of health and safety risks to users and patients.

NOTE 2 A design and development control process suitable for IVD manufacturers and other developers of nucleic acid-based *in vitro* diagnostic examination procedures is described in ISO 13485:2003, 7.3.

The requirements of ISO 13485:2003, 4.2.3 apply to the control of documents and records pertaining to development of the examination procedure.

NOTE 3 Design, development, and documentation control requirements do not apply to research activities conducted prior to the start of development of an IVD medical device or examination procedure.

Instructions for use, including operator manuals where appropriate, shall be prepared according to the requirements of ISO 18113-1:2009, ISO 18113-2:2009, and ISO 18113-3:2009. Each of the steps required to perform an examination, the required quality assurance measures, and requirements for laboratory facilities and utilities shall be described in the instructions for use.

EXAMPLE Specimen collection and processing, nucleic acid extraction, nucleic acid amplification, and detection and identification of nucleic acids of the target microbial pathogen, laboratory design, work flow, and laboratory practices.

In addition, the instructions for use shall contain a description of the nucleic acid sequences that may be processed in the examination procedure.

If appropriate, the medical application and clinical utility of examination results shall be explained in the instructions for use.

4.1.2 Implementation and use in the medical laboratory

The medical laboratory shall document its examination procedures and maintain records of its decisions and actions. The nucleic acid sequences processed by the laboratory shall be documented.

The requirements of ISO 15189:2012, 4.3 and 4.13 apply to the control of documents and records.

EXAMPLE Equipment set up and maintenance, calibration traceability and measurement uncertainty, biological reference intervals, quality control procedures and criteria.

NOTE A quality management system suitable for medical laboratories and other users of nucleic acid-based *in vitro* diagnostic examination procedures is described in ISO 15189.

A medical laboratory that implements a validated nucleic acid-based IVD examination procedure without modification shall verify its performance before it is introduced into routine use. The requirements of ISO 15189:2012, 5.5.1.2 apply.

Subsequent modifications to a validated examination process shall be validated. The requirements of ISO 15189:2012, 5.5.1.3 apply.

4.2 Specimen collection, transport, and storage conditions

Requirements for specimen collection, transport, and storage shall be specified in the instructions for use. The requirements of ISO 18113-2:2009 apply.

NOTE Guidelines for "Collection, Transports, Preparation and Storage of specimens for Molecular methods" are found in CLSI MM13-A2[22] and JCCLS MM5-A1.[28]

Particular attention shall be given to potential effects of specimen collection, transport, and storage on the steps required to prepare the specimen for nucleic acid extraction.

EXAMPLE Specimen type, specimen container, criteria for specimen acceptability, specimen handling procedure to minimize changes due to nucleic acid loss or contamination, amount required, additives required, transport conditions, storage conditions, stability factors, and precautions.

The medical laboratory shall incorporate the requirements for specimen collection, transport, and storage as instructions in corresponding sections of the sample collection manual.

4.3 Selection of nucleic acid targets and sequences

Criteria for selection of nucleic acid targets and sequences shall be specified in the instructions for use.

The target sequences shall be identified in terms of the nucleic acid of the target microbial pathogen.

EXAMPLE Genomic or plasmid DNA, transcripts such as mRNA or rRNA or cDNA of viruses, genomic RNA, or 16S or 23S rRNA of bacteria.

The target sequences shall be evaluated for their degree of homology with other organisms, where appropriate, using a publicly accessible nucleic acid sequence database.

EXAMPLE International Nucleotide Sequence Database Collaboration, which comprises the European Molecular Biology Laboratory (EMBL) Nucleotide Archive,[30] the DNA DataBank of Japan (DDBJ),[31] and GenBank at the US National Center for Biotechnology Information.[32] These three organizations exchange data on a daily basis.

NOTE Only a small number of the total known bacterial and viral species have been sequenced. Some sequences have not been verified.

The laboratory shall periodically check the sequence database(s) for updates to determine the need to modify the laboratory's technical procedures.

If prior evidence is not available to show that the target sequence is universally present in the target microbial pathogen, a sufficient number of strains shall be examined to provide statistically valid evidence of its universal presence in the target pathogen.

EXAMPLE If 5 % of the organisms lack the target sequence, then examination of at least 60 % of the strains is required to have a 95 % chance of detecting at least one isolate lacking the target sequence.

4.4 Selection of primers or primer sequences

The procedure to select the primer sequences shall be designed to detect the target microbial pathogen.

Appropriate design criteria may include length, G and C content, melting temperature, avoiding secondary structures, and noncomplementarity.[21]

Evaluation of several sets of primers may be required in order to achieve the expected performance.

NOTE Software is available to help with primer design.

4.5 Nucleic acid preparation and stability

Conditions that ensure adequate preparation and stability of the nucleic acid after extraction shall be defined, validated, and documented in the instructions for use.

The purity, integrity, and yield of nucleic acid extracted from the sample shall be adequate for the intended uses. If sufficient nucleic acid is not present in the sample, the extraction shall be repeated using the same sample or another sample shall be collected for extraction.

NOTE For information on nucleic acid preparation and stability, refer to ISO 21571:2005, 5.2.[2]

The laboratory shall prepare and store the nucleic acid extract according to the instructions for use to ensure that the purity, integrity and stability are adequate to perform the examination.

4.6 Nucleic acid amplification

Several different amplification methods are available.[38][40] Amplification of the target sequence occurs *in vitro* through a reaction catalysed by a DNA polymerase in the presence of oligonucleotide primers and deoxynucleoside triphosphates in a defined reaction buffer. For RNA-based organisms, reverse transcription is required prior to amplification of the target sequence.

Precautions shall be taken to prevent the reaction mixture from containing polymerase inhibitors.

NOTE For information on nucleic acid amplification methods, refer to ISO 21569:2005, 7.3.[1]

4.7 Nucleic acid detection and identification

The method for detection of the amplified target shall be appropriate to demonstrate the presence, absence, or the characteristics of the genetic element under study, relative to appropriate controls and within the detection limits of the examination procedure used and the sample examined.

The method selected for detection of the amplified target shall be validated for its ability to detect the amplified target sequences. Validation shall include comparison to a reference measurement procedure or other recognized examination procedure.

NOTE For information on nucleic acid detection and identification methods, refer to ISO 21569:2005, 7.6.[1]

4.8 Reagent stability and storage conditions

Reagent stability and storage conditions shall be determined, validated and specified in the instructions for use. The requirements of ISO 23640:2011 and ISO 18113-2:2009 apply.

Particular attention shall be given to

a) establishment of reagent shelf life, including transport conditions suitable to ensure that product specifications are maintained,

b) establishment of stability of the reagent in use after the first opening of the primary container,

   EXAMPLE On-board stability, stability after reconstitution, open vial/bottle stability.

c) monitoring of stability of reagents already placed on the market or distributed for use, and

d) verification of stability specifications after modifications of the reagent that might affect stability.

Where appropriate according to the instructions for use, the laboratory may aliquot the reaction solutions required for the analytical method to avoid subjecting them to repeated freeze–thaw cycles and to reduce the chance of contamination.

5 Performance characteristics

5.1 General requirements

5.1.1 Design and development

The analytical and clinical cut-off values and performance characteristics pertaining to the detection and identification of microbial pathogens shall be determined and validated for the intended uses and documented in the instructions for use. The requirements of ISO 18113-2:2009, 7.16 apply.

Unless otherwise specified, the performance characteristics shall represent the performance of the entire examination process.

The analytical performance characteristics to be specified shall include analytical sensitivity, detection limit, analytical specificity (cross-reactivity and interference), and, where appropriate, trueness and measurement precision. The statistical method and rationale used to establish the analytical performance characteristics shall be described and documented.

The clinical performance characteristics to be specified shall depend on the intended uses of the examination results.

EXAMPLE Diagnosis of a particular infectious disease.

The control procedures and control materials necessary to maintain the required performance characteristics shall be determined, validated, and specified in the instructions for use. The requirements of ISO 18113-2:2009, 7.13 apply.

5.1.2 Implementation and use in the medical laboratory

The performance characteristics relevant to the intended uses shall be verified by the medical laboratory before the examination procedure is placed into routine use. The requirements of ISO 15189:2012, 5.5.1.2 apply.

NOTE 1 For guidelines to evaluate the performance of qualitative examination procedures, refer to CLSI EP 12:2008.[15]

The measurement uncertainty shall be adequate for the intended uses.

NOTE 2 For information regarding the determination of measurement uncertainty, refer to JCGM 100:2008 ("GUM"),[12] Technical report No. 1/2006, EUROLAB, 2006[27] and CLSI EP29-A: 2012.[19]

NOTE 3 The expanded measurement uncertainty can be used to delineate indeterminate "grey zones" around the cut-off values.

Quality assurance procedures shall be established by the laboratory, taking into consideration the recommendations in the instructions for use. The rationale for the selection of control materials and control procedures shall be documented.

NOTE 4 For guidelines to establish quality assurance procedures, refer to ISO 24276:2006.[6]

The relevant performance characteristics shall be re-evaluated after any changes that affect the examination procedure, and shall be reviewed at periodic intervals. The frequency of re-evaluation should be based on the risks associated with incorrect results.

EXAMPLE Calibration, reagent change, equipment maintenance, new operator.

5.2 Specific requirements

5.2.1 Cut-off values

The analytical cut-off values for detection and identification of the target microbial pathogen shall be determined based on the requirements of the intended uses.

NOTE 1 For information regarding the use of Receiver Operating Characteristic curves to determine cut-off values, refer to CLSI EP24-A2:2012.[18]

Cut-off values shall be verified when the examination procedure is initially placed in service, and shall be verified with every change in lot, instrument maintenance, and at periodic intervals appropriate for the intended uses.

5.2.2 Detection Limit

The minimum amount of target DNA sequence that can be detected in a sample with 95 % confidence shall be determined.

NOTE For information regarding determination of the detection limit, refer to CLSI EP17-A2: 2012.[16]

The detection limit shall be verified when the examination procedure is initially placed in service, and shall be verified with every change in lot (e.g. new master mix), instrument maintenance, and at periodic intervals appropriate for the intended uses.

5.2.3 Analytical specificity

The analytical specificity for the target microbial pathogen shall be determined for the entire examination procedure.

Cross-reactivity shall be checked with a panel of relevant organisms. Interference shall be checked with a panel of relevant endogenous and exogenous substances.

The false-positive rate shall be determined with a sufficient number of samples with no target sequence (negative specimens).

The specimen processing procedures shall be evaluated for interference with the examination procedure, from the nucleic acid extraction step to detection and identification of the microbial pathogen.

NOTE 1 For information on “analytical specificity”, refer to ISO 18113-1:2009, A.2.6.

NOTE 2 For guidelines to evaluate analytical interference and cross-reactivity, refer to CLSI EP07-A2:2005.[14]

5.2.4 Measurement precision

The relevant precision characteristics for the measurement results shall be determined, where appropriate.

EXAMPLE Repeatability, intermediate precision, reproducibility.

NOTE 1 Where qualitative results (e.g. positive and negative results) are being determined from numerical measurement results, measurement precision near the clinical cut-off values is a relevant performance characteristic.

NOTE 2 For general principles regarding the evaluation of measurement precision and guidelines to determine relevant components of measurement precision, refer to CLSI EP05:2004,[13] ISO 5725-1,[7] ISO 5725-2,[8] ISO 5725-3,[9] and ISO/TR 22971:2005.[10]

NOTE 3 For information pertaining to measurement precision claims (repeatability, intermediate precision and reproducibility) in IVD medical device labelling, refer to ISO 18113-1:2009, A.2.3.

Measurement precision shall be verified when the examination procedure is initially placed in service, and shall be monitored on an ongoing basis. Suitable control materials may be used to monitor measurement precision. The monitoring interval shall be appropriate for the intended uses.

NOTE 4 For guidance for the use of repeatability, reproducibility, and trueness estimates in the determination of measurement uncertainty, refer to ISO 21748:2010.[4]

5.2.5 Clinical performance

The clinical performance characteristics of the examination procedure relevant to the intended uses shall be established based on clinical evidence.

Since clinical performance depends on the prevalence of the target condition in the population being evaluated, a description of the intended population and the statistical methods shall accompany any claims of clinical performance.

EXAMPLE Clinical sensitivity, clinical specificity, clinical accuracy.

NOTE 1 For information about clinical evidence and clinical performance, refer to GHTF SG5/N 6:2012.[25]

NOTE 2 For information about clinical accuracy, refer to the STARD Initiative.[33]

NOTE 3 For information about scientific validity determination and clinical performance evaluation for IVD medical devices, refer to GHTF SG5/N7:2012.[26]

The clinical evidence shall include studies of specimens from patients who have been diagnosed with the infection and those who do not have the infection. The number of samples used in the clinical studies shall be statistically justified.

NOTE 4 Evaluation of clinical accuracy can be based on a combination of analytical and clinical criteria to determine if the infection of interest is present or absent.

5.3 Quality control and quality assurance procedures

5.3.1 Control materials

Appropriate controls shall be identified and used to reduce the likelihood of producing an incorrect result due to inadequate performance of the examination procedure. An assessment of the potential for error and the appropriate controls to minimize the potential for error shall be conducted and the rationale for the selection of control materials and procedures shall be documented.

EXAMPLES

a) Positive controls (target nucleic acid present) to monitor detectability of the target pathogen.

b) Negative controls (target nucleic acid absent) to monitor analytical specificity.

c) Blank controls to verify the absence of contamination of reagents or unacceptable background signal, where appropriate.

Internal controls may be added to the primary sample matrix (serum, plasma, culture media), and extracted, amplified, analysed, and detected independent of a target. The internal control shall be processed in the same tube with the target.

Considering the difficulty inherent in validating and using an internal control, well-known and well-characterized external controls may alternatively be run in parallel with the samples under examination.

EXAMPLE Use of positive and negative patient samples as positive and negative controls, respectively.

5.3.2 Medical laboratory design and workflow

The design and layout of medical laboratories used to perform nucleic acid-based examination procedures shall take into account the special needs for these procedures. Appropriate precautions shall be taken to minimize the risk of contamination leading to a false-positive result (see Clause 6).

EXAMPLES

a) Separate work areas for reagent preparation, specimen preparation including DNA and RNA isolation, and target detection.

   NOTE Physical separation through the use of different rooms is the most effective and therefore preferred way to separate work areas.[6]

b) Personnel traffic and forward workflow from the sample preparation area to the sample analysis area. Written laboratory procedures (see 5.3.3) to reduce the opportunity for contamination of the reagent preparation and specimen preparation areas by laboratory equipment, clothing, and personnel exposed to the post-amplification analysis area.

NOTE General instructions and requirements are found in ISO 24276:2006, 6.4.[6]

5.3.3 Medical laboratory practices

Processes within the medical laboratory shall be designed and implemented to prevent contamination and ensure quality examinations.

The laboratory procedures shall include at a minimum the following precautions, where applicable, to reduce the likelihood of contamination. Requirements to use separate equipment and supplies for reagent preparation, sample preparation, amplification, and detection do not apply to steps that are performed on the same instrument.

a) Use separate equipment and supplies for reagent preparation, specimen preparation, and post-amplification analysis.

b) Change gloves between each step, or more often as needed, and when entering or re-entering separate areas.

c) Dedicate laboratory coats to specific areas and change laboratory coats when entering or leaving each area

d) Add non-sample components to the reaction tubes before adding the sample

e) Keep reagent tubes capped unless in use

f) For post amplification manipulation of PCR amplicons (e.g. gel electrophoresis or DNA sequencing), pulse spin PCR reaction tubes before uncapping

g) Do not transfer laboratory protocol or record books from an area containing sample nucleic acid or amplicon to an area that does not contain sample nucleic acid or amplicon

h) Do not transfer gel electrophoresis photographs printed in an area containing sample nucleic acid or amplicon to an area that does not contain sample nucleic acid or amplicon for filing and do not take the camera's memory card from the post-amplification area to another area for printing

NOTE Gel images generated in an area containing sample nucleic acid or amplicon can be transferred electronically for printing.

5.3.4 Commercial equipment (including software)

Equipment intended to perform nucleic acid-based examinations, including software necessary to perform the analysis, shall be installed, qualified, calibrated, and maintained according to the

manufacturer's instructions for use and documented laboratory procedures. The requirements of ISO 15189:2012, 5.3 apply.

Where applicable, integration of laboratory instruments into existing IT infrastructure shall be verified. The requirements of ISO 15189:2012, 5.10 apply.

EXAMPLE Connectivity to databases, bioinformatic functions, etc.

5.3.5 Medical laboratory personnel

Personnel assigned to perform nucleic acid-based examinations shall be qualified and trained and shall receive the continuing education needed to maintain competency. The requirements of ISO 15189:2012, 5.1 apply.

5.3.6 Quality assurance procedures

Appropriate quality assurance procedures shall be implemented to ensure the quality of nucleic acid-based examination results. The requirements of ISO 15189:2012, 5.6 apply

In particular, the quality assurance procedures shall be designed to minimize false-positive and false-negative results. Control measures shall be implemented to prevent potential failures of the three main stages of nucleic acid-based *in vitro* diagnostic examinations: (1) sample preparation and nucleic acid extraction; (2) nucleic acid amplification; and (3) nucleic acid detection and identification (see Clause 6).

5.4 Reporting of results

Appropriate procedures shall be implemented to ensure the timely reporting of results. The requirements of ISO 15189:2012, 5.8 apply.

In addition to the requirements of ISO 15189:2012, 5.8.3, the report shall include the following:

a) nucleic acid target;

b) date of sampling and date of measurement;

c) examination results;

d) description of the procedure used, including amplification and extraction;

e) controls used;

f) identification of source and type of sample;

g) critical results and/or interpretive comments;

h) limitations of the examination procedure.

The reference interval for the qualitative detection and identification of a microbial pathogen shall be stated.

EXAMPLE "Not detected" or "Less than the detection limit".

6 Risk management

6.1 General

Organizations that develop, manufacture, distribute, and/or perform nucleic acid-based *in vitro* diagnostic examination procedures each have a role in managing the health and safety risks to patients, users and other individuals.

The risk management process shall be established, documented, and maintained as an ongoing process for identifying hazards associated with using the *in vitro* diagnostic examination procedure, estimating and evaluating the associated risks, controlling these risks within acceptable limits, and monitoring the effectiveness of the risk controls.

Risk management responsibilities and activities shall be planned and approved by top management according to established procedures.

NOTE A risk management plan can include risk acceptability criteria; responsibilities and authorities; risk analysis and evaluation; risk control verification activities; risk management review requirements; risk-benefit analysis; and activities related to monitoring risks from ongoing use of the examination procedure.

6.2 Design and development risk management

The risks associated with using the nucleic acid-based *in vitro* diagnostic examination procedure for microbial pathogens shall be analysed during development of the examination procedure based on the intended uses, and the estimated risks shall be evaluated for acceptability. These activities shall be performed according to documented procedures.

The risks to be considered shall include at a minimum

— risks to patients from false-negative and false-positive examination results, and

— risks to laboratory workers and other users associated with the performance of examination procedures, including biological hazards.

Potential failure modes and use errors that could occur at each of the three main stages of nucleic acid based examinations shall be analysed to identify hazards and hazardous situations and estimate the risks. Unacceptable risks shall either be reduced to acceptable levels or be reduced as far as reasonably practicable. A risk-benefit analysis may be conducted to determine risk acceptability.

NOTE 1 For a risk management process suitable for IVD manufacturers and other developers of *in vitro* diagnostic examination procedures, refer to ISO 14971:2007. Guidelines for IVD medical devices are described in Annex H.

NOTE 2 For guidance for implementing risk management principles and activities within a quality management system, refer to GHTF/SG3/N15R8:2007.[23]

The instructions for use accompanying the examination procedure shall include information to enable medical laboratories to control the risks to patients from false-negative and false-positive examination results, along with recommendations to control the risks where appropriate. Any significant residual risks shall be disclosed.

NOTE 3 Risk control measures can include quality control activities, warnings, specific instructions for use, preventive maintenance, etc.

6.3 Medical laboratory risk management

The nucleic acid-based examination process shall be analysed by the medical laboratory to identify potential failure modes, use errors, hazards and hazardous situations and the risks to patients and laboratory workers shall be estimated and evaluated for acceptability prior to implementation of the examination process.

Risk control measures necessary to protect patients and laboratory workers from the identified hazards and hazardous situations shall be verified and implemented according to the risk management plan.

The medical laboratory shall ensure the safety and protection of laboratory staff and service personnel. The requirements of ISO 15190:2003 apply.

Note 1 The general principles and risk management practices described in ISO 14971:2007 and Reference [23] can also be applied to medical laboratories.

Note 2 For general guidance for reduction of laboratory error, refer to ISO/TS 22367:2008.[11]

Note 3 For information about quality control planning based upon risk management principles, refer to CLSI EP23.[17]

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