
Diagnostični preskusni sistemi in vitro - Zahteve in priporočila za odkrivanje koronavirusa (SARS-CoV-2) z metodami amplifikacije nukleinskih kislin (ISO/TS 5798:2022)

In vitro diagnostic test systems - Requirements and recommendations for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by nucleic acid amplification methods (ISO/TS 5798:2022)

In-vitro-Diagnostika-Systeme - Anforderungen und Empfehlungen für Qualitätsverfahren für den Nachweis des Coronavirus 2 des Schweren Akuten Respiratorischen Syndroms (SARS-CoV-2) mittels Nukleinsäureamplifikation (ISO/TS 5798:2022)

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Systèmes d'essai pour diagnostic in vitro - Exigences et recommandations pour la détection du coronavirus 2 associé au syndrome respiratoire aigu sévère (SARS-CoV-2) par des méthodes d'amplification des acides nucléiques (ISO/TS 5798:2022)

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**In vitro diagnostic test systems —
Requirements and recommendations
for detection of severe acute
respiratory syndrome coronavirus 2
(SARS-CoV-2) by nucleic acid
amplification methods**

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 212, *Clinical laboratory testing and in vitro diagnostic test systems*, in collaboration with Technical Committee ISO/TC 276, *Biotechnology*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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Introduction

Coronaviruses are enveloped RNA viruses that are broadly distributed in the animal kingdom. They have been identified in humans, other mammals, and birds. Coronaviruses were named because the spike proteins known to facilitate viral attachment and cell entry appear like a halo on the virus surface when viewed under an electron microscope. Coronaviruses are roughly spherical with a diameter ranging from 118 nm to 136 nm. The coronavirus genome, which ranges from 26 kb to 32 kb, is the largest among all RNA viruses, including RNA viruses that have segmented genomes. Until 2019, six coronaviruses have been associated with human diseases:

- severe acute respiratory syndrome-related coronavirus (SARS-CoV),
- Middle East respiratory syndrome coronavirus (MERS-CoV),
- human coronavirus 229E (HCoV-229E),
- human coronavirus OC43 (HCoV-OC43),
- human coronavirus NL63 (HCoV-NL63), and
- human coronavirus HKU1 (HCoV-HKU1)[1].

In 2019, a cluster of patients presenting with a respiratory disease were shown, by sequencing, to be infected with a novel coronavirus[2]. The coronavirus associated with this cluster was subsequently named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses[3]. SARS-CoV-2 is the seventh coronavirus known to infect humans. The disease caused by SARS-CoV-2 was designated as coronavirus infectious disease 2019 (COVID-19) by the World Health Organization (WHO)[4].

The host range for SARS-CoV-2 is not yet fully defined. SARS-CoV-2 is a beta-coronavirus. The receptor for SARS-CoV-2 is the angiotensin-converting enzyme 2 (ACE2). ACE2 is a cell-surface, zinc-binding carboxypeptidase involved in regulation of cardiac function and blood pressure. It is expressed in epithelial cells of the lung and the small intestine, which are the primary targets of SARS-CoV-2, as well as the heart, kidney, and other tissues.

SARS-CoV-2 replicates in the upper and lower respiratory tracts and is transmitted by droplets and aerosols and most likely other contact with asymptomatic and symptomatic infected persons. The basic reproduction number (R_0) of the original variant is between 2 and 3, but significantly more contagious variants have developed. The median incubation period is 5,7 (range 2 to 14) days[5]. Similarly to SARS and MERS, superspreading events have been reported, with a dispersion parameter (kappa) estimated at 0,1. Most infections are uncomplicated, and 5 % to 10 % of patients are hospitalized mainly due to pneumonia with severe inflammation. However, complications include respiratory and multiorgan failures. Risk factors for the complicated disease increase with age and include hypertension, diabetes, chronic cardiovascular and chronic pulmonary diseases, and immunodeficiency.

Clinical management of COVID-19 and control of infections and spread of SARS-CoV-2 require effective and efficient in vitro diagnostics. There are a number of tests and kits in use for the detection of SARS-CoV-2 and the number of methods will continue to increase. Acceptable design, development and establishment of quality SARS-CoV-2 diagnostics based on nucleic acid detection methods is critical to ensure COVID-19 infection control. Establishing indices for conducting comprehensive quality evaluation of these methods and kits both during development and in routine application will ensure the accuracy of the test results and support epidemic prevention and control. This document provides requirements and recommendations to consider for the quality practice of SARS-CoV-2 nucleic acid amplification methods.

In vitro diagnostic test systems — Requirements and recommendations for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by nucleic acid amplification methods

1 Scope

This document provides requirements and recommendations for the design, development, verification, validation and implementation of analytical tests for detecting the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) using nucleic acid amplification. It addresses pre-examination, examination and post-examination process steps for human specimens.

This document is applicable to medical laboratories. It is also intended to be used by in vitro diagnostic developers and manufacturers, as well as by institutions and organizations supporting SARS-CoV-2 research and diagnostics.

This document does not apply to environmental samples.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

severe acute respiratory syndrome coronavirus 2

SARS-CoV-2

virus that causes coronavirus infectious disease 2019 (COVID-19)

3.2

specimen

primary sample

discrete portion of a body fluid, breath, hair or tissue taken for examination, study or analysis of one or more quantities or properties assumed to apply for the whole

Note 1 to entry: The Global Harmonisation Task Force (GHTF) uses the term specimen in its harmonized guidance documents to mean a sample of biological origin intended for examination by a medical laboratory.

Note 2 to entry: In some countries, the term “specimen” is used instead of “primary sample” (or a subsample of it), which is the sample prepared for sending to, or as received by, the laboratory and which is intended for examination.

[SOURCE: ISO 15189:2012, 3.16^[6] modified — Note 2 to entry was removed and Note 3 to entry was renumbered as Note 2 to entry.]

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3.3

sample

one or more parts taken from a *primary sample* (3.2)

EXAMPLE A volume of serum taken from a larger volume of serum.

[SOURCE: ISO 15189:2012, 3.24^[6]]

3.4

reverse transcription

RT

process of making complementary DNA [cDNA (3.6)] from an RNA (3.20) *template* (3.22), using the enzymatic activity of a reverse transcriptase associated with one or more oligonucleotide primers under a suitable set of conditions

[SOURCE: ISO 16577:2016, 3.180^[7], modified — Replaced “DNA” with “complementary DNA (cDNA)”.]

3.5

deoxyribonucleic acid

DNA

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

[SOURCE: ISO 22174:2005, 3.1.2^[8]]

3.6

complementary DNA

cDNA

single-stranded DNA (3.5), complementary to a given RNA (3.20) and synthesised in the presence of reverse transcriptase to serve as a *template* (3.22) for DNA amplification

[SOURCE: ISO 20395:2019, 3.5^[9]]

3.7

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

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

[SOURCE: ISO 18113-1:2009, A.3.4^[10]]

3.8

limit of detection

LOD

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 0,05, given a probability of 0,05 of falsely claiming its presence

[SOURCE: ISO/IEC Guide 99:2007, 4.18^[11], modified — “ β , given a probability α ” was replaced by “0,05, given a probability of 0,05” and Notes 1 to 3 to entry were deleted.]

3.9

verification

provision of objective evidence that a given item fulfils specified requirements

[SOURCE: ISO/IEC Guide 99:2007, 2.44^[11], modified — EXAMPLES 1 to 3 and Notes 1 to 6 to entry were deleted.]

3.10 validation

confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

Note 1 to entry: The word “validated” is used to designate the corresponding status.

[SOURCE: ISO 9000:2015, 3.8.13^[12], modified — Notes 1 and 3 to entry were deleted and Note 2 to entry was renamed Note 1 to entry.]

3.11 amplicon

specific *DNA* (3.5) fragment produced by a DNA-amplification technology, such as the *polymerase chain reaction* (*PCR*) (3.12)

[SOURCE: ISO 13495:2013, 3.3.1^[13]]

3.12 polymerase chain reaction PCR

enzymatic procedure which allows in vitro amplification of *DNA* (3.5) or *RNA* (3.20)

[SOURCE: ISO 22174:2005, 3.4.1^[8], modified — “or RNA” added to the end of the definition and “in vitro” has been unitalicized in accordance with the ISO House Style.]

3.13 reference material

material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties

[SOURCE: ISO/IEC Guide 99:2007, 5.13^[11], modified — Notes 1 to 8 to entry and EXAMPLES 1 to 5 were deleted.]

3.14 pseudo-virus

virus or virus-like particle that can integrate the envelope glycoprotein of another virus to form a virus with an exogenous viral envelope, and the genome retains the characteristics of the retrovirus itself

3.15 digital PCR dPCR

procedure in which nucleic acid *templates* (3.22) are distributed across multiple partitions of nominally equivalent volume, such that some partitions contain *template* and others do not, followed by *PCR* (3.12) amplification of target sequences and detection of specific *PCR* products, providing a count of the number of partitions with a positive and negative signal for the target template

Note 1 to entry: Nucleic acid target sequences are assumed to be randomly and independently distributed across the partitions during the partitioning process.

Note 2 to entry: The count of positive and negative partitions is normally based on end point detection of *PCR* products following thermal cycling, however real-time *qPCR* (3.16) monitoring of *PCR* product accumulation is additionally possible for some dPCR platforms.

[SOURCE: ISO 20395:2019, 3.10^[9]]

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3.16 quantitative real-time PCR qPCR

enzymatic procedure which combines the in vitro amplification of specific *DNA* (3.5) or *RNA* (3.20) segments with the detection and quantification of specific *PCR* (3.12) products during the amplification process

Note 1 to entry: While the *PCR* is producing copies of the relevant *DNA* sequence, the fluorescent marker fluoresces in direct proportion to the amount of *DNA* present, which can theoretically be back-calculated to infer the original amount of that particular *DNA* present in a *sample* (3.3) prior to initiation of *PCR*.

[SOURCE: ISO 20395:2019, 3.25^[9], modified — “RNA” was added.]

3.17 quantification cycle

C_q
quantitative real-time PCR (qPCR) (3.16) cycle at which the fluorescence from the reaction crosses a specified threshold level at which the signal can be distinguished from background levels

Note 1 to entry: Quantification cycle is a generic term which includes cycle threshold (C_t), crossing point (C_p), take off point and all other instrument specific terms referring to the fractional cycle which is proportional to the concentration of target in the qPCR assay.

Note 2 to entry: The quantification cycle is based either on a threshold applied to all *samples* (3.3) or on a regression analysis of the signal, for each sample.

Note 3 to entry: The quantification cycle is a measure with poor reproducibility and cannot be used when comparing kit performance.

Note 4 to entry: Laboratory based considerations sometimes lead to selection of a cut-off for the cycle number. The cut-off cannot be chosen not to have a detrimental influence on available *limit of detection* (3.8).

Note 5 to entry: C_q does not apply for *digital PCR* (3.15) and isothermal amplification methods.

[SOURCE: ISO 20395:2019, 3.8^[9], modified — Notes 3 to 5 to entry have been added.]

3.18 clinical specificity diagnostic specificity

ability of an in vitro diagnostic examination procedure to recognize the absence of a target marker associated with a particular disease or condition

Note 1 to entry: Also defined as “percent negativity” in *samples* (3.3) 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 $100 \times \frac{\text{number of true negative values (TN)}}{\text{sum of the number of true negative plus the number of false positive (FP) values, or } 100 \times \text{TN} / (\text{TN} + \text{FP})$. 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.

[SOURCE: ISO 18113-1:2009, A.3.16^[10]]

3.19 clinical sensitivity diagnostic sensitivity

ability of an in vitro diagnostic examination procedure to identify the presence of a target marker associated with a particular disease or condition

Note 1 to entry: Also defined as “percent positivity” in *samples* (3.3) 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 $100 \times \frac{\text{number of true positive values (TP)}}{\text{number of true positive values (TP)} + \text{number of false negative values (FN)}}$, or $100 \times \frac{\text{TP}}{\text{TP} + \text{FN}}$. 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.

[SOURCE: ISO 18113-1:2009, A.3.15^[10]]

3.20

ribonucleic acid

RNA

polymer of ribonucleotides occurring in a double-stranded or single-stranded form

[SOURCE: ISO 22174:2005, 3.1.3^[8]]

3.21

calibrator

measurement standard used for calibration

[SOURCE: ISO 20395:2019, 3.4^[9], modified — Note 1 to entry and the EXAMPLE were deleted.]

3.22

template

strand of *DNA* (3.5) or *RNA* (3.21) that specifies the base sequence of a newly synthesized strand of *DNA* or *RNA*, the two strands being complementary

[SOURCE: ISO 16577:2016, 3.206^[7]]

3.23

saliva

whole saliva

bio-fluid of the mouth composed mainly of secretion originating from the three major salivary glands (parotids, submandibular and sublingual glands) and from salivary glands present in the oral cavity

[SOURCE: ISO 4307:2021, 3.15^[14]]

3.24

reverse transcription polymerase chain reaction

RT-PCR

process that combines *RT* (3.4) and *PCR* (3.12) to allow amplification of *cDNA* (3.6) target as a route to detect *RNA* (3.20) templates (3.22)

Note 1 to entry: This can be conducted using various formats. A popular approach uses real time *PCR* instrumentation which simultaneously conducts the *PCR* and the analysis; this is described as reverse transcription quantitative PCR [RT-*qPCR* (3.16)].

Note 2 to entry: Adapted from ISO 20395:2019, 3.31^[9].

3.25

in vitro diagnostic medical device

IVD medical device

device, whether used alone or in combination, intended by the manufacturer for the in vitro examination of *specimens* (3.2) derived from the human body solely or principally to provide information for diagnostic, monitoring or compatibility purposes and including reagents, *calibrators* (3.21), control materials, specimen receptacles, software, and related instruments or apparatus or other articles

[SOURCE: ISO 17511:2020, 3.21^[15]]

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3.26

pre-examination processes

processes that include preparation and identification of the patient, collection of the primary *specimen(s)* (3.2), transportation to and within the medical laboratory, and isolation of *RNA* (3.20)

Note 1 to entry: Pre-analysis or pre-analytics are synonymous with pre-examination.

Note 2 to entry: Adapted from ISO 15189:2012, 3.15^[6].

3.27

limit of quantification

LOQ

lowest concentration or content of the analyte of interest per defined amount of *matrix* (3.31) that can be measured with reasonable statistical certainty consistently under the experimental conditions specified in the method

Note 1 to entry: Generally expressed in terms of the signal or measurement (true) value that will produce estimates having a specified relative standard deviation (RSD).

[SOURCE: ISO 16577:2016, 3.91^[7]]

3.28

positive PCR control

reliable source of well-characterized positive *sample* (3.3) material, containing intact target nucleic acid sequences for *PCR* (3.12)

[SOURCE: ISO 16577:2016, 3.150^[7], modified — Note 1 to entry was deleted.]

3.29

internal inhibition control

material acting as an internal control and obtained during the amplification reaction of the target fragment by adding *DNA* (3.5) or primers

Note 1 to entry: This material is clearly different from the target fragment.

Note 2 to entry: Adapted from ISO 16577:2016, 3.82^[7].

3.30

laboratory developed test

LDT

test developed (or modified) and used within a single laboratory to carry out testing on *samples* (3.3), where the results are intended to assist in clinical diagnosis or to be used in making decisions concerning clinical management

Note 1 to entry: Laboratory developed test needs to be validated for its intended use before putting into service.

Note 2 to entry: Adapted from ISO 17822:2020, 3.23^[16].

3.31

matrix

components of a material system, except the analyte

[SOURCE: ISO 15193:2009, 3.6^[17]]

3.32

matrix effect

influence of a property of the *sample* (3.3), independent of the presence of the analyte, on the measurement and thereby on the measured quantity value

[SOURCE: ISO 15194:2009, 3.7^[18], modified — Notes 1 to 2 to entry and the EXAMPLE were deleted.]

3.33

no template control

NTC

control reaction containing all reagents except the extracted test *sample* (3.3) *template* (3.22) nucleic acid

Note 1 to entry: This control is used to demonstrate the absence of contaminating nucleic acids. Instead of the template DNA, for example, a corresponding volume of nucleic acid free water is added to the reaction. The term “PCR reagent control” is also sometimes used.

[SOURCE: ISO 20395:2019, 3.20^[9]]

3.34

loop-mediated isothermal amplification

LAMP

strategy for achieving isothermal *DNA* (3.5) amplification by utilizing two or three uniquely designed primer sets and a polymerase with high strand displacement activity

[SOURCE: ISO 16577:2016, 3.94^[7]]

4 Overview

4.1 SARS-CoV-2

4.1.1 General

The process of SARS-CoV-2 molecular detection testing using the nucleic acid amplification test (NAAT) typically includes pre-examination and examination steps. The pre-examination steps include collection of clinical specimens, transport, storage, sample lysis, and nucleic acid extraction and concentration. Examination steps include reverse transcription (cDNA synthesis) and an appropriate amplification method. In addition, post-examination steps such as management of waste and reporting of the test results are included.

Quality attributes for the NAAT-based detection processes include, but are not limited to, evaluation of the performance of a suitable extraction procedure, evaluation of test reagents to meet minimum test criteria, a comprehensive evaluation of the analytical specificity, limit of detection (LOD) of the assay, and evaluation of the stability of the reagents.

The technical procedure to evaluate the quality attributes is shown in [Figure 1](#), including the whole process evaluation and the key analytic performance evaluation.

NOTE The nucleic acid extraction part of quality evaluation is not always needed for NAATs where only one nucleic acid extraction method is used or when the extraction method is an integral part of the workflow.