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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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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

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

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Foreword

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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*.

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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.

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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

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.]

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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 × 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.

[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.

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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.

[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.]

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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.

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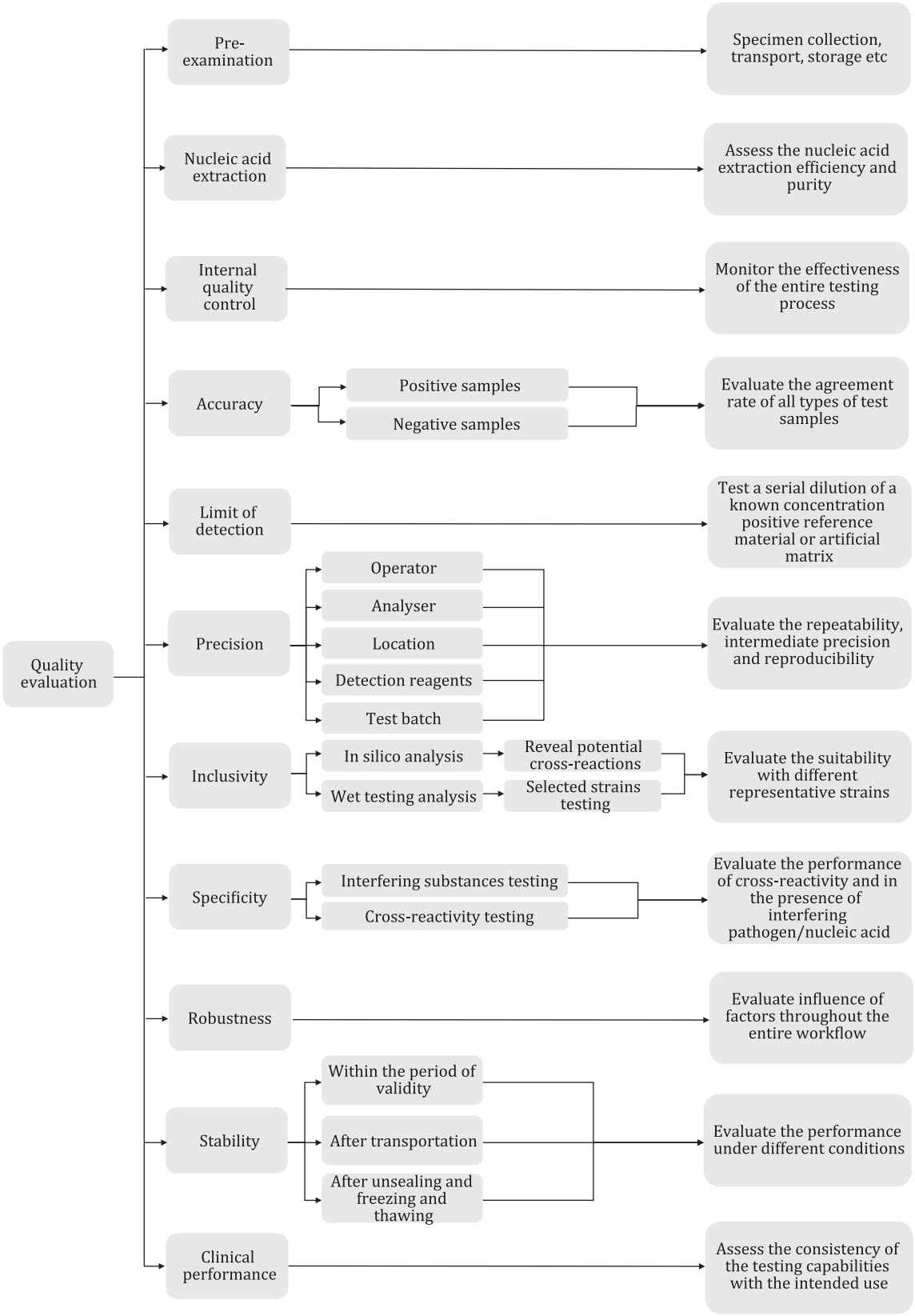

Figure 1 — Workflow of quality evaluation of SARS-CoV-2 detection method based on the nucleic acid amplification test (NAAT)

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4.1.2 Pre-examination

For the detection of SARS-CoV-2, during the pre-examination work process, the following general considerations should be taken into account:

a) Appropriate personal protective equipment (PPE) should be used.

b) Specimen type selection: SARS-CoV-2 mainly infects the respiratory system; specimen selection should be determined with reference to the characteristics of SARS-CoV-2-related exposure or infection.

c) Specimen collection: depending on the selected specimen types, clinical specimens should be obtained according to standardized sampling requirements.

d) Specimen packaging: specimen packaging should take into account appropriate biosafety practices.

e) Specimen transport and storage: during the transport and storage process, the impact on the degradation of viral nucleic acid should be taken into account.

f) Inactivation of SARS-CoV-2: before testing in the laboratory, initial processing (before inactivation) of all specimens should take place in a validated biosafety cabinet (BSC) or primary containment device. If initial procedures involve manipulation of a primary specimen (e.g. dilution with inactivating reagent), they should be included in assay verification and validation.

NOTE Further detailed information on pre-examination parameters can be found in 6.5.1.

4.1.3 Examination — Overview

4.1.3.1 General

During laboratory testing of SARS-CoV-2 nucleic acid, the following general considerations should be taken into account:

a) Appropriate PPE should be used for all examinations.

b) Separate equipment, single use disposables, or both should be used for all activities to avoid cross-contamination.

c) Sample extraction, reaction reagent preparation, and amplicon handling should be conducted in separate laboratory rooms.

The need for separate rooms can be somewhat reduced by use of commercially available assays, closed-tube methods, and automated instruments. The fully automated methods require only one room or dedicated zone for laboratories using only commercially available kit-based assays. Closed-tube methods are methodologies where the amplification and analysis are performed in a single tube without the need to transfer the PCR products for further analysis.

d) Unless used for post-amplification steps, opening tube caps should be avoided as much as possible.

e) To avoid cross-contamination, it is recommended to avoid movement of the instrument or sharing equipment in different work areas.

f) For detection methods using conventional NAAT techniques, partition requirements for a NAAT laboratory should be strictly followed when performing the tests.

g) The dUTP and Uracil-DNA Glycosylase (UDG) may be included in the reaction mix to eliminate amplicon contamination.

h) Temporary storage and disposal of waste during sample testing should be considered.

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