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TSS-20

In vitro diagnostic medical devices used for the qualitative detection of SARS-CoV-2 nucleic acid

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Abbreviations

ANOVA	analysis of variance
CI	confidence interval
Ct	cycle threshold
EUL	emergency use listing
IFU	instructions for use
IS	International Standard
IVD	in vitro diagnostic
IU	international units
LOD	limit of detection
NA	nucleic acid
NAT	nucleic acid amplification technology
NP	nasopharyngeal
OP	oropharyngeal
POC	point of care
RT-PCR	reverse transcriptase polymerase chain reaction
ROC	receiver operated curve
TGS	Technical guidance series
TSS	Technical specifications series
US FDA	U.S. Food and Drug Administration
UTM	universal transport media
VOC	variants of concern
VOI	variants of interest
VTM	viral transport media
WHO	World Health Organization

A. Introduction

The purpose of this document is to provide technical guidance to in vitro diagnostic (IVD) medical device manufacturers that intend to seek WHO prequalification of qualitative, multiplex (dual or triple viral target) nucleic acid amplification technology (NAT) for the detection of SARS-CoV-2.

For the purpose of this document, the verbal forms used follow the usage described below:

- "shall" indicates that the manufacturer is required to comply with the technical specifications
- "should" indicates that the manufacturer is recommended to comply with the technical specifications, but it is not a requirement
- "may" indicates that the technical specifications are suggested methods to undertake the testing, but not requirements

A documented justification and rationale shall be provided by the manufacturer when the WHO prequalification submission does not comply with the required technical specifications outlined in this document.

Where possible, WHO analytical and clinical performance study requirements are aligned with published guidance, standards and/or regulatory documents. Although references to source documents are provided, in some cases WHO prequalification has additional requirements.

For WHO prequalification purposes, manufacturers shall provide evidence in support of the clinical performance of an IVD to demonstrate that reasonable steps have been taken to ensure that a properly manufactured IVD, being correctly operated in the hands of the intended user, will detect the target analyte consistently and fulfil its indications for use.

WHO prequalification requirements summarized in this document do not extend to the demonstration of clinical utility, i.e. the effectiveness and/or benefits of an IVD, relative to and/or in combination with other measures, as a tool to inform clinical intervention in a given population or healthcare setting. To demonstrate clinical utility, a separate set of studies is required. Clinical utility studies usually inform programmatic strategy and are thus the responsibility of programme managers, ministries of health and other related bodies in individual WHO Member States. Such studies do not fall under the scope of WHO prequalification.

B. How to apply

Prequalification guidance documents for compiling and preparing a prequalification application may differ to the recommendations to support an application under the Emergency Use Listing /EUL) procedure. Manufacturers, who are applying for prequalification with a product, that has been EUL listed, are required to follow prequalification guidance. The submission of the dossier must be according to TSS requirements and prequalification dossier instructions "Instructions for compilation of a product dossier – IMDRF ToC (PQDx_018, v5 November 2022)" (insert ref):

C. Other guidance documents

This document should be read in conjunction with other WHO guidance documentation, including:

- Technical Guidance Series for WHO Prequalification – Diagnostic Assessment available at https://extranet.who.int/pqweb/vitro-diagnostics/technical-guidance-series

- WHO Prequalification "Instructions for Compilation of a Product Dossier", WHO document PQDx_018. (1)
- Target product profiles for priority diagnostics to support response to the COVID-19 pandemic v.1.0 28 September,2020 Geneva, Switzerland (2)
- Diagnostic testing for SARS-CoV-2 Interim guidance 11 September 2020 (3)
- Laboratory biosafety guidance related to coronavirus disease (COVID-19): interim guidance, 13 May 2020 (4)

D. Performance principles for WHO prequalification

D.1 Intended use

An IVD submitted for WHO prequalification assessment shall be accompanied by a sufficiently detailed intended use statement. This should allow an understanding of at least the following:

- the type of assay
- what the IVD medical device measures or detects;
- its function (e.g. screening, diagnosis or aid to diagnosis,
- the specific disorder, condition or risk factor of interest that it is intended to detect, define or differentiate;
- whether or not it includes automated components or is intended to be used with automated instruments;
- what the IVD medical device reports (e.g., qualitative test, semi-quantitative);
- the type of specimen(s) (e.g. nasopharyngeal (NP)swabs, oropharyngeal (OP) swabs, anterior or mid turbinate nasal swabs, sputum, etc) required including the specimen collection method, storage/transport medium (e.g. swab in viral transport media (VTM) universal transport media (UTM)); and
- target population (on whom the IVD medical device is used).
- The intended use environment and the intended user (e.g. trained laboratory professionals trained in the techniques of real time RT-PCR and IVD procedures)
- Any limitations to the intended use (e.g. identification or restrictions regarding age groups or other limiting characteristics).

D.2 Diversity of specimen types, users and testing environments and impact on required studies

For WHO prequalification submission, clinical performance studies shall be conducted using the specimen types (e.g. NP or OP swabs, anterior or mid turbinate nasal swabs, sputum, etc) that are claimed in the instructions for use (IFU).

Prequalified SARS-CoV-2 NAT assays in low- and middle-income countries are likely to be used by a range of users in different geographical regions:

- laboratory professionals[1] either in centralised testing laboratories or at/near POC,
- laboratory professionals in health care settings not experienced in nucleic acid testing,
- health professionals trained in the use of the test at or near POC.

Depending on the intended use of an IVD, analytical and clinical performance studies shall be designed to consider not only the diversity of knowledge and skills across the population of IVD users, but also the

[1]Medical technologists, medical laboratory technicians or similar, who have received a formal professional or paraprofessional certification or tertiary education degree.

likely operational settings in which testing will occur. It is a manufacturer's responsibility to ensure that the risk assessment for an IVD reflects the intended operational settings, including laboratory or service delivery complexity, user expertise, training received and test population.

D.3 Applicability of supporting evidence to IVD under review

Analytical and clinical performance studies shall be undertaken using the specific, final (locked-down) version of the assay intended to be submitted for WHO prequalification assessment. For WHO prequalification, design lock-down is the date that final documentation, including quality control and quality assurance specifications, is signed off and the finalized method is stated in the IFU. Where this is not possible, a justification shall be provided, and additional supporting evidence may also be required. This may occur in the case of minor variations to design where no impact on performance has been demonstrated (see WHO document PQDx_121 Reportable Changes to a WHO Prequalified In Vitro Diagnostic Medical Device) (5). If the method section of the IFU has been changed in any way, both the study protocol provided to laboratory for clinical performance studies as outlined in Part 2 of this document and that in the final version of the IFU intended for users shall be provided with the submission for WHO prequalification assessment.

The version of the IFU used for verification and validation studies submitted for WHO prequalification assessment shall be stated. If the test procedure in the IFU is changed in any way after completing performance verification and validation studies the change(s) shall be reported to WHO, including a rationale for the change, and an explanation of why the study results support the claimed performance.

Specific information is provided in this document for the minimum numbers of lots required for each study. Where more than one lot is required, each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents, representative of routine manufacture. It is a manufacturer's responsibility to ensure, via risk analysis of its IVD that the minimum numbers of lots chosen for estimating performance characteristics considers the variability in performance likely to arise from the interlot diversity of critical components and their formulation or from changes that could occur during the assigned shelf life of the IVD. Differences found between lots during the analytical and clinical performance studies shall be reported. All instrumentation required when running the assay (from specimen processing to result interpretation) shall be specified and validated for the product under review.

The true clinical status (presence of absence of active SARS-CoV-2 infection) status shall be determined using a suitable molecular reference method. For WHO purposes this should be a nucleic acid amplification test (NAT) that currently is at a developed stage of technical capability based on the relevant consolidated findings of science, technology and experience (commonly referred to as state of the art). Justification for the choice of method, shall be provided. Manufacturers intending to submit clinical studies that were conducted and assessed as part of the WHO EUL procedure are recommended to contact WHO in advance of the submission.

Estimation (and reporting) of IVD performance shall include the rate of invalid test results and the two-sided 95% confidence interval around the estimated values for key performance metrics. The cause of invalid results should be reported if available. Data should be presented in a clear and understandable format.

For analytical performance studies described in part 1 it may be also possible to carefully design protocols that will generate useful data for more than one of the required studies, provided the specific criteria for each requirement are met by the study (e.g. number of replicates, concentration of analyte, specimen

types, etc.). For example, precision testing and whole system failure testing could be combined in a single study. Studies which may fall in this category are indicated in the appropriate sections of part 1.

If the validation of specimens (chapter 3.05.02) shows equivalency between specimen types, some analytical performance studies (as indicated in this document) may use a representative specimen type only. However, if no equivalence between specimen types is shown, validation shall be conducted in all specimen types where indicated in the TSS.

Clinical performance studies shall be based on testing human specimens only sourced from population cohorts reflective of the intended use. Independent of the outcome of the equivalency study (chapter 3.05.02), all claimed specimens types need to be considered in the clinical performance study, unless otherwise stated in part 2. The use of well-characterised repository specimens and panels may be acceptable if they are relevant to the IVD under assessment, taking into consideration:

- storage conditions (e.g. including age of the specimen, temperature logs, freeze-thaw cycles if applicable);
- the stability of the nucleic acid target;
- selection bias;
- information on specimen collection date, date of symptom onset, etc. as required for the clinical study.

Studies that comprise the testing of left-over specimens by research and development staff at a manufacturer's facility shall not, on their own, be considered sufficient to meet the clinical performance study requirements summarized in this document.

E. Table of Requirements

WHO requires that a product dossier is submitted in the "Table of Contents" (ToC) format, described in the International Medical Device Regulators Forum (IMDRF) document IMDRF/RPS WG/N13 FINAL:2019 (Edition 3) (6). In the tables below, the chapters and subheadings are labelled and numbered according to IMDRF ToC format. As the IMDRF ToC is comprehensive in nature, not all subheadings are required for WHO prequalification and are excluded. As a result, the subheading numbering in the tables below is not always continuous (e.g., 3.1.1, 3.1.3, etc). This has been done to maintain consistency between sections required in a product dossier for WHO prequalification assessment and the corresponding numbering defined in the IMDRF ToC format.

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IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirements	Source Documents
3.05.01 Stability of specimen(s)			
Specimen collection, storage and transport	1. Identify the different specimen types (e.g. NP swabs, OP swabs, sputum, nasal swabs) that can be used with the IVD. 2. If recommended transport/storage conditions in the IFU are outside the standard recommendations for swab collected respiratory virus specimens in commercially available and validated transport media (e.g., UTM, VTM),then evidence for stability of the specimen shall be provided. 3. For transport/storage media which are proprietary to the manufacturer and are provided with the product, evidence of the recommended storage conditions shall be provided 4. Real time studies shall be conducted for each claimed specimen type, including transport/storage media that is provided by the manufacturer if applicable, taking into account: • Storage conditions (for example, duration at different temperatures, temperature limits, freeze/thaw cycles etc.) • Transport conditions • Intended use (see note 1) • Specimen collection (if provided with the test)(5. The testing panel shall contain (see note 3, 4) • A minimum of 10 discrete weak positive specimens approximately 3 x limit of detection (LOD) (see note 2) for each specimen type 6. Testing shall be conducted in 1 lot	1. Evidence shall be provided which validates the maximum allowable time between specimen collection and its processing or addition to the IVD in the setting where testing takes place 2. The LOD is defined as the minimum number of target sequences in a sample volume that can be detected in 95% of tests. (chapter 3.05.05) 3. Contrived specimens may be used, prepared in discrete matrices that simulate the specimen type 4. In case the use of archived specimens is considered for Section 4.02.03 of this document, evidence of stability in the conditions in which the specimens have been stored shall be demonstrated e.g. by re-testing a subset of specimens with a suitable NAT to verify that the same result is obtained compared to the sample result prior to storage 5. Acceptance criteria shall confirm that claimed specimen types transported, processed and stored under recommended conditions will give expected results • The manufacturer shall define what the acceptable deviation is when reporting their results in the study report 6. Unless all specimens are expected to be processed as fresh samples within a specified time frame, the IVD performance shall be established for each storage condition at the beginning and end of the stated period	TGS 3 (7) CDC (9)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirements	Source Documents
3.05.02 Validation of specimens			
Demonstration of equivalence between specimen types	The relationship between IVD performance in claimed specimen types shall be established: 1. At least 25 positive and 25 negative specimens shall be tested for each claimed specimen type (see notes 1-4) 2. 1 replicate of each specimen of each specimen type shall be tested and the results compared between matrices 3. Testing shall be conducted in 1 lot	1. If multiple specimen types are claimed (e.g., NP swab, OP swab, nasal swab, sputum, etc) a matrix equivalency study should be conducted to establish the relationship between specimen type and IVD performance 2. Specimens should be chosen that have low to moderate concentrations of the analyte 3. Contrived specimens may be used 4. LOD studies chapter 3.05.05 may also contribute to evidence regarding equivalence of specimen types 5. The established relationship between IVD performance in claimed specimen types shall be considered in the design of subsequent studies. For example, if a manufacturer can demonstrate equivalency between two or more matrices or specimen types, only one representative specimen type/matrix needs to be tested in the following analytical studies: section 3.05.04 Precision, 3.05.05b VOC sensitivity, 3.06.04a Robustness, and 3.06.05 Stability of the IVD	
3.05.03 Metrological traceability of calibrators and control material values			
Metrological traceability of calibrators and control material values	1. The manufacturer should demonstrate that the controls provided in the kit and reference material used in the validation studies are traceable to the WHO International Standard (IS) for SARS-CoV-2 RNA (see note 1) or a secondary standard calibrated against it	1. The version of the IS used shall be stated 2. For any reference materials from an outside source (e.g. commercial source), a detailed report from the supplier shall be provided, and its relationship to the IS should be established	WHO TRS 1004 (10) TGS-6 (11)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirements	Source Documents
3.05.04 Accuracy of measurement			
3.05.04.02 Precision (Repeatability & reproducibility)	1. Both repeatability (see note 1) and reproducibility (see note 2) shall be estimated using panels with defined analyte levels. 4. The members of the repeatability and reproducibility testing panel shall include (see note 5, 6): • 1 x negative specimen • 1 x low positive specimens with a concentration of analyte (approx.. 3x LOD) • 2 x moderately positive specimens with a concentration of analyte (approx. 5-7x LOD) 5. Each panel member shall be tested: • in 5 replicates • using 3 different lots (see notes 7, 8) • over 5 days (not necessarily consecutive) with 1 run in that day (alternating morning/afternoon) • at each of 3 different testing sites (see note 9) • using 1 operator/site (see note 8) • by operators representative of intended users • unassisted • using only those materials provided with the IVD (e.g. IFU, labels and other instructional material) 6. Where relevant, multiple instruments may be used for the testing 7. All claimed specimen types shall be tested unless equivalency has been demonstrated in chapter 3.05.02	1. Within run 2. Between -run, -lot, -day, -site, operator 3. A run is defined depending on the IVD's throughput: if the platform can accommodate all specimens in a single run, i.e. in the same test plate, the specimens will be run together. If the assay can only accommodate a smaller set or a single specimen(s), a run will be defined as a testing session carried out on the same instrument/module on the same day. 4. Precision shall be determined utilizing the entire test system (specimen processing, nucleic acid extraction, detection) 5. Specimens with target levels of analyte may be contrived. 6. The testing panel should be the same for all operators, lots and sites. If there is no equivalence between claimed specimen types, then the impact that this will have on each subsequent performance claim shall be fully understood and described 7. Each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents, representative of routine manufacture. 8. If operators are considered a significant source of test result variation (for example, with tests that have a significant proportion of manual manipulations), then at least 2 operators/site shall be used 9. To understand irregularities in results obtained, at least 2 lots should be tested at each of the 3 testing sites. 10. The number of invalid tests shall be reported.	CLSI EP05-A3 (12) CLSI EP12 (13)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirements	Source Documents
		11. Results shall be statistically analysed by ANOVA or other methods to identify and isolate the sources and extent of any variance. In addition, the percentage of correctly-identified, incorrectly-identified and invalid results shall be tabulated for each specimen and be separately stratified according to each of site, lot, etc. 12. Defining acceptable coefficients of variation as <5% is strongly encouraged 13. Acceptance criteria shall be defined that describe the maximum amount by which the Ct value deviate before acceptable performance is said to be affected. 14. The repeatability and reproducibility studies can be combined into a single study with appropriate study design to facilitate robust statistical analysis. 15. Alternative methods used to establish repeatability and reproducibility performance of the assay shall be discussed with WHO in advance of dossier submission	
3.05.05 Analytical sensitivity			
3.05.05a Limit of detection	1. The LOD shall be estimated as the concentration of SARS-CoV-2 RNA detectable 95% of the time. 2. Testing 20-24 replicates of at least 8 serial 0.5$\log_{10}$ dilutions of suitable biological reference materials (see note 2). The serial dilutions shall be chosen so that the RNA concentrations span the LOD of the IVD (see notes 3, 4) 3. The replicate testing shall be conducted on 3 different days (see note 5) (8 replicate tests on each day) • Using 2 lots • At least 2 dilution series shall be tested	1. The LOD of the IVD shall be determined utilizing the entire test system from specimen processing, nucleic acid extraction, to detection 2. The WHO International standard for SARS-CoV-2 RNA or a secondary standard calibrated against it shall be used. The version of the WHO International Standard shall be stated. 3. To inform the concentrations of the specimens that will span the LOD of the IVD, a tentative LOD can be established by preparing limiting dilutions of the spiked material followed by nucleic acid extraction and 3-5 replicate measurements of each.	CLSI EP17-A2 (14)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirements	Source Documents
	4. The LOD shall be determined for all claimed specimen types irrespective of any equivalence that has been determined.	4. For testing the 20-24 replicates of the dilution series, nucleic acid shall be extracted for each replicate test. 5. For low through-put instruments, the number of testing days may be increased 6. LOD shall be estimated by determining the 95% LOD with 95% confidence intervals (CI) (e.g. by probit analysis) 7. The LOD of the IVD shall be expressed in IU/mL 8. The nucleic acid extraction/purification method, extraction platform (if applicable) and elution volume, PCR instrument and cycling conditions shall be provided.	
3.05.05b VOC sensitivity	The potential impact of emerging variants on the IVD performance shall be evaluated if *in silico* analyses reveals any mismatches: 1. Testing shall be conducted using at least 4 specimens of the predominant variants circulating in the populations for which claims are made, if available 2. In addition, testing shall be conducted using at least 2 specimens of all the other claimed variants, if available 3. Testing dilutions of the specimens at low LOD (approx. 2-3x LOD) and medium concentration (approx. 5-7 LOD) • 10 replicates/dilution • Using 2 lots 4. All claimed specimen types shall be tested unless equivalency has been demonstrated in chapter 3.05.02 (see note 3)	1. The lineage, NCBI GenBank or GISAID accession number shall be provided 2. If cell culture derived virus is used, a detailed report from the supplier shall be provided and the following information must be included as a minimum: source, passage history, virus titre (PFU/mL or TCID50/mL); further, the virus stock shall be characterized by PCR and the copy/mL value provided 3. The dilution factor and number of serial dilutions of the characterized SARS-CoV-2 specimen that were tested to determine the LOD shall be provided 4. Contrived specimens may be used	NCBI GenBank (15) GISAID (16) WHO Variant website (17)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirements	Source Documents
3.05.06 Analytical specificity			
3.05.06a Potentially interfering substances and medical conditions	The potential for false results (false negative and false positive results) arising from interference by the substances/conditions listed below shall be determined (see note 1, 2, 3): 1. by testing SARS-CoV-2-negative specimens (see note 4) and low/weak positive specimens (negative specimens spiked with SARS-CoV-2 at 3x LOD) 2. A minimum of 100 specimens shall be tested 3. testing shall be conducted using a minimum of 5–10 specimens per substance/condition 4. In triplicate Using only 1 claimed specimen type (see note 5)	1. The risk assessment conducted for an IVD shall identify substances at medically relevant levels for which the potential for interference can reasonably be expected for the analyte being detected in the areas of intended use and not simply rely on published lists of such compounds and conditions which might be of limited relevance in resource limited settings • By conducting appropriate risk assessment, testing can be conducted on specimens spiked with the substances/ conditions identified as likely to be significant and testing of potentially irrelevant substances/conditions avoided • Under some circumstances stringent risk evaluation may eliminate the requirement to test some of the items in the lists but any such decision shall be documented in any submissions to WHO and taken into account in the risk-benefit statements 2. Any observed interference shall be further investigated and performance limitations of the IVD reported in the IFU 3. Results shall be reported with respect to each condition and not be reported as an aggregate of the total number of specimens tested in the study 4. Prior to spiking, samples should be confirmed to be SARS-CoV-2-negative prior to testing with a suitable RT-PCR assay 5. If non-respiratory clinical specimen types are claimed, additional substances may need to be considered. Please contact WHO for further information 6. For NAT that use conventional PCR and/or well-established extraction methods prior to reverse transcription and amplification, (e.g. Boom method and column based extraction methods),	CLSI EP07 (18) CLSI EP37 (19) European common specifications (20)
Endogenous and Exogenous	The interference of endogenous and exogenous substances in one claimed specimen type on the performance of the device shall be investigated. 1. Endogenous and exogenous substances shall be spiked at the highest levels found in individuals. 2. A list of the interfering substances tested and the concentrations used shall be provided., 3. Substances to be tested include: • Mucin: Bovine submaxillary gland, type 1-5 • Human blood • Nasal sprays or drops • Nasal corticosteroids • Nasal gel • Anaesthetic and analgesic throat lozenges, • Anti-viral drugs		

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirements	Source Documents
	• Antibiotic nasal ointment • Systemic antibacterial medications	interference studies are not necessarily required for respiratory specimens.	
Cross-reactivity	1. The manufacturer shall determine the potential for false results arising from cross-reactivity with: • Near neighbour species/strains or • Organisms whose infection produces symptoms similar to those observed at the onset of covid-19, and • Pathogenic microflora that may be present in the claimed specimens type 2. Testing shall include, where possible, at least 3-5 each of: • Human coronavirus 229E • Human coronavirus OC43, Human coronavirus HKU1, Human coronavirus NL63, SARS-Coronavirus • MERS-Coronavirus • Adenovirus (e.g. C1 Ad. 71), Human Metapneumovirus (hMPV), Parainfluenza virus 1-4, Influenza A, Influenza B • Enterovirus (e.g. EV68), Respiratory syncytial virus, Rhinovirus, *Chlamydia pneumoniae, Haemophilus influenzae,* Legionella pneumophila, Mycobacterium tuberculosis • Streptococcus pneumoniae • Streptococcus pyogenes • Bordetella pertussis • Mycoplasma pneumoniae	1. Negative clinical specimens may be spiked with the organism of interest to a high concentration (a minimum of 10^5 plaque forming units/mL for viruses and 10^6 colony forming units/mL for bacteria) 2. Any observed cross-reactivity shall be further investigated and performance limitations of the IVD reported in the IFU 3. Specimens shall be confirmed to be SARS-CoV-2-negative by RT-PCR prior to testing 4. Omissions from actual laboratory testing shall be supported by a well-documented justification that includes a due diligence attempt to obtain the organisms (and/or purified nucleic acid). *In silico* analysis may be performed as part of the risk assessment • *In silico* analysis should include multiple representative strains from GenBank sequence database[2] for each organism • The full sequence of each organism should be analysed • If *in silico* analysis reveals other potential cross-reactants (i.e., ≥80% homology between one of the primers or the probe to any of the sequences of listed potential cross reactants), carefully review the alignments and determine based on the positions of the homologous stretches and mismatches if additional cross-reactivity and/or interference (please refer to microbial interference studies) laboratory testing will be required to rule out cross-reactivity or interference of that organism that may affect the performance of your device	

[2] https://www.ncbi.nlm.nih.gov/genbank/

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirements	Source Documents
	• Pneumocystis jirovecii (PJP) • Candida albicans • Pseudomonas aeruginosa • Staphylococcus epidermis • Streptococcus salivarius • Pooled human nasal wash – to represent diverse microbial flora in the human respiratory tract 3. Samples should be tested in triplicate 4. Using one claimed specimen type (or artificial matrix)	• In these circumstances if laboratory testing is omitted you should include an explanation as to why *in silico* generated data is not clinically relevant (irrelevant isolate, location/extent of match within primer/probe, etc.), or why the performance of your test would not be impacted 5. If non-respiratory clinical specimen types are claimed for diagnostic use with your device additional organisms may need to be considered.	
Microbial interference	If *in silico* analysis reveals ≥80% homology between the microorganisms nucleic acid and the test primers/probe(s), there could be interference with amplification of the target gene (even in the absence of cross-reactivity). In this case, the following study shall be considered: 1. A microbial interference study with SARS-cov-2 and the microorganisms that the test primers/ probe(s) have homology to 2. Specimens shall be spiked at a low (3 x LOD) SARS-CoV-2 concentration and a high interferent level, to represent the worst-case scenario, with a minimum of 3 replicates 3. If interference is observed at the level tested, an additional titration study should be performed to determine the highest microorganism interferent level the SARS-CoV-2 test can tolerate	1. Microbial interference studies aim at demonstrating that false negatives for SARS-CoV-2 will not occur in presence of other microorganisms 2. Otherwise explain why the *in-silico* results are irrelevant 3.	
3.05.09 Validation of assay cut-off			
Validation of assay cut-off	1. The assay cut-off shall be validated by testing the following testing panel (see note 2):	1. Test samples chosen shall display a range of Ct values that are representative of routine clinical cases.	

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirements	Source Documents
	• 100 SARS-CoV-2 positive clinical specimens representative of low, medium and high viral loads (see note 1) • 1000 SARS-CoV-2 negative specimens • The testing panel shall include 10 positive and 10 negative specimens close to the cut-off • The testing panel should contain different variants to the extent possible 2. The most challenging specimen type shall be tested 3. The manufacturer shall justify the positioning of the cut-off and describe the algorithm/method used to set the cut-off for the test, or in cases where the cut-off is set for each run or set of tests, the manufacturer shall describe the algorithm/method specified in the IFU or used by the instrument to set the cut-off	2. Contrived specimens (high positive clinical specimens diluted in an appropriate negative matrix) may be used if natural clinical specimens of the required concentration are not available 3. Specimens used to establish the cut-off shall be different from the specimens used to validate the cut-off (so that the two processes are independent)	
3.05.10 Validation of the assay procedure			
3.05.10a Validation of primer and probe choice	1. Evidence supporting the choice of critical reagents (primers and probes sequences) shall be provided	1. A rationale for selection of primers and probes including target genes and specific sequences used shall be provided, including: • Justification for alignments made to generate consensus sequences or best-fit modifications made to existent sequences e.g. to permit maximum homology to several strains, and • Information on size, GC content, melting temperatures, hairpin or other secondary structures if any, and the nucleotide position on the genome map of the primers and probes 2. The potential impact of genetic variations with focus on mutations or deletions associated with VOC and variants of interests (VOI) shall be assessed. Laboratory testing to understand the impact on	NCBI GenBank (15) GISAID (16)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirements	Source Documents
		performance shall be conducted if mismatches are identified by in silico analysis	
3.05.10b Procedural control	1. The product shall include an internal control: • If an endogenous internal control (housekeeping gene) is used as part of the assay design, an acceptable range of Ct values should be determined for each specimen type. • If an exogenous internal control is used as part of the assay, evidence for the acceptable Ct range shall be provided 2. The product shall include a positive and negative control. • The concentration of the positive control shall be near the LOD and shall at a minimum control for amplification and detection of the target RNA	1. The design of the internal control shall be risk based, and a justification provided	
3.05.10c Whole system failure rate	1. The potential for false negative results in low positive specimens shall be determined: 2. The testing panel shall be randomized and contain (see note 1): • 10 specimens containing SARS-CoV-2 (approx. 3 x LOD) 3. The testing panel shall be tested: • On 5 consecutive days • Using 1 lot • With 1 user The whole system failure shall be determined for the most challenging specimen type claimed.	1. This may be conducted as part of precision studies if the minimum number of replicates are met 2. Replicate contrived specimens should be prepared using a single specimen diluted in the appropriate matrix	European common specifications (20)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirements	Source Documents
3.06 Other Studies			
3.06.02 Software/Firmware/Programmed or programmable medical devices			
3.06.02.08 Software Verification and Validation	1. Software validation reports shall be available for submission if requested (see note 1)	1. Software validation to include as a minimum: • Verification of built-in fail-safe. • Verification of alert mechanisms. • Verification of quantitative/semi-quantitative result detection & interpretation. • Evidence to demonstrate that appropriate error codes are provided to the end user	IEC 62304:2006 / Amd 1:2015 (21) U.S FDA (22)
3.06.04 Usability/human factors studies			
3.06.04a Flex studies/ robustness	1. Evidence is required to demonstrate that the conditions recommended in the IFU are validated and how they were verified. 2. Robustness (flex) studies shall be designed to challenge the system under conditions of stress to identify potential device deficiencies, including failures, and determine the robustness of the product. 3. The influence of the following factors on expected results (both detected and non-detected) shall be considered as applicable (see note 1, 2, 3, 4): • Specimen and/or reagent volume (see note 4) • IVD instrument sturdiness (including the effect of non-level work surface) • Lighting, humidity and barometric pressure (simulating high altitude)	1. Refer to WHO document PQDx_018 for other flex studies that may be relevant, taking into consideration the broad range of operational and environmental conditions consistent with intended use 2. The risk assessment conducted for an IVD shall identify factors which have potential to affect the performance of the assay 3. The factors should be investigated in ways that not only reflect, but also exceed, likely operating conditions in lower- and middle-income countries so that the limitations of the device can be understood 4. Additional factors may be relevant for point-of-care devices or devices requiring significant manual interventions (e.g. manual extraction). These factors may include errors during sample collection, sample handling and loading, and handling of relevant test components after sample application 5. For the purposes of this document, ruggedness means the ability to resist environmental shocks of a variety of kinds	PQDx_018 (1) IEC 62366-1:2015 (23)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirements	Source Documents
	• Handling contamination (e.g. from latex, powder, hand lotion, sweat, and/or soap, etc.) • Operating temperature 4. Instrumentation (both extraction and amplification) including: • Ruggedness (including the effect of vibration from other instruments) (see note 5) • Impact of dust and mould on componentry (e.g. optics) • Impact of power/voltage fluctuation 5. Testing to be performed in 1 lot 6. The specimen panel shall contain: • 1 negative specimen • 1 specimen spiked with SARS-CoV-2 (approx. 3 x LOD) 7. Where different specimen types are claimed, flex studies shall use the most challenging specimen type	6. Robustness testing generally takes the form of statistically designed experiments to evaluate the effect of simultaneous "small but deliberate changes" in method parameters 7. Since assay and analyser parameters are locked down in a closed system and cannot be changed, there should be evidence that these parameters have been optimized	
3.06.04b Usability: Label comprehension (including IFU) study	1. Testing of subjects to assess ability of intended users to correctly comprehend key messages from packaging and labelling • Understanding key warnings, limitations and/or restrictions, including correct use of self-collection methods and equipment • Proper test procedure • Test result interpretation 2. Studies shall include at least 15 intended users, users including those whose native language may not be the language of the IFU if necessary, to demonstrate comprehension of key messages in the user population	3. IFU and labelling should be clear and easy to understand. Use of pictorial instructional material is encouraged	IEC 62366-1:2015 (23) European Parliament IVD regulations (30)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirements	Source Documents
3.06.04c Usability: Results interpretation study	1. Intended users shall interpret a range of test results to assess their ability to correctly interpret pre-determined test results and error messages. 2. Testing subjects to consist of at least 15 intended users including those whose native language may not be the language of the IFU	3. Study group may include subjects recruited as part of the label comprehension study	
3.06.04d Carry-over contamination	The potential for false positive results due to carry-over shall be investigated. 1. The testing panel shall contain 40 alternating high-positive (≥ 10^6 IU/mL or copies/mL) and negative specimens (see note 1) 2. Only 1 specimen type should be used. The most challenging specimen type shall be chosen as the test specimen. 3. The panel shall be tested: • In at least 5 different runs • On 3 different days • At least 2 users • Using 1 lot 4. For testing platforms that can only accommodate a single specimen, testing shall be conducted on a single instrument: • At least 4 tests per run • Using alternating high-positive (≥ 10^6 IU/mL or copies/mL) and negative specimens • A total of 10 runs • At least 2 users Using 1 lot	1. Either clinical specimens (individual or pooled) or high titre cell culture derived virus diluted in the appropriate matrix shall be used 2. Whether the IVD is a microtiter plate based system or a single use device, appropriate studies that will detect carry-over contamination should be designed	European common specifications (20) Haeckel R (24)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirements	Source Documents
3.06.05 Stability of the IVD			
3.06.05.01 Claimed Shelf-life & 3.06.05.03 Shipping Stability	1. Stability studies shall be evaluated for the shelf life of the test kit. The following conditions shall be investigated: • Conditions to mimic extremes of conditions (temperature, humidity, pressure) exposed to during transport • Minimum and maximum storage temperature and humidity range 2. The reagents shall be subjected to simulated shipping conditions prior to placing them into the shelf-life studies 3. All kit configurations should be tested (or a rationale provided if not) 4. At least 3 lots shall be tested (see note 4, 5) 5. The stability testing panel will consist of the following contrived specimens (see note 3): • 1 SARS-CoV-2 low positive specimen (approx. 3 x LOD) • 1 medium positive specimen (5 – 7 x LOD) • 1 negative specimen 6. Each panel member shall be tested in triplicate at each time point/condition (see note 8) 7. All claimed specimen type shall be tested unless specimen equivalency has been demonstrated in chapter 3.05.02	1. When more than one part of the genome is targeted by primers, each region shall be monitored separately during stability evaluation 2. If the assay contains more than one of each primer/probe combination, then each primer/probe combination needs to be assessed for stability 3. Specimens with target levels of analyte can be contrived by spiking an appropriate negative matrix with SARS-CoV-2 positive material with a known concentration SARS-CoV-2 RNA 4. Each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents, representative of routine manufacture 5. The number of invalid tests with each kit lot shall be reported 6. Claims for stability shall be based on the second-last successful data point from the least stable lot, with, if lots are different, a statistical analysis showing that the majority of lots will be expected to meet the claimed life. For example: for testing conducted at 3, 6, 9, 12 and 15 months, if stability was observed at 15 months, then the maximum stability claim can be 12 months 7. Statistically designed experiments should be involved to allow evaluation of any interactions between environmental conditions. 8. Ct values of each replicate, condition and time point shall be provided 9. Determination of shipping stability shall be performed using simulated extreme stress conditions, ensuring that application of those conditions is consistent and controlled. Transport stress shall be applied before assigning lots to shelf-life studies to mimic the real-life situation	ISO 23640:2011 (25) CLSI EP25 (26) TGS 2 (27) WHO Annex TGS 2 (28) ASTM D4169 (29)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirements	Source Documents
		10. Accelerated studies do not replace the need for real time studies 11. Multiple instruments may be used to allow simultaneous testing at each time point	
3.06.05.02 In-use stability (open pack or open vial stability)	1. Minimum of 1 lot shall be tested using a stability testing panel composed of (see note 3): • 1 negative specimen • 1 low SARS-CoV-2 positive specimen (approx. 3 x LOD) and • 1 medium positive specimen (5 – 7 x LOD) 2. Replicate testing of each panel member (see note 1) 3. Testing shall be conducted using the most challenging specimen type 4. All labile components (e.g. bulk volume buffers, single use reagent vials, sealed cartridges, control materials etc.) shall be evaluated 5. On-board stability shall be evaluated for an IVD used with an instrument	1. Justification for the number of replicates shall be based on the stability study set up, statistical analysis of the data and a prior knowledge of the assay's performance 2. In-use stability of labile components shall be conducted using components in their final configuration 3. Contrived specimens may be used 4. Consideration shall be given to operating temperature, humidity range and allowable freeze-thaw cycles of reagents/controls, as applicable	

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Aspect	Testing requirements	Notes on testing requirements	Source Documents
4.02.03 Device Specific Clinical Studies			
4.02.03a General requirements for clinical sensitivity and specificity studies	Testing shall be conducted: 1. On specimens from all sections of the population for which claims are made 2. If the intended use includes a claim for testing of asymptomatic individuals, this population shall be included in the clinical study. 3. Using specimens from at least 2 different, geographically diverse regions by a variety of intended users representing relevant intended use settings and at different test settings 4. Using at least 2 lots (see chapter D.3) 5. The specimens shall be tested with an RT-PCR test (reference method) 6. Testing of all claimed specimen types is required (see note 1, 11, 14) 7. Specimens with discrepant results shall be further evaluated. Where possible, follow-up testing shall be done (see note 3) 8. The procedure for selection of study specimens, how these represent an intended use population and how bias has been addressed shall be clearly described.	1. Clinical performance shall be established using specimens that correspond directly to claims made in the IFU 2. Specimens with unexpected results but which otherwise meet selection criteria for a study, shall not be systematically excluded from analysis 3. Discrepant results should be resolved as much as possible, however performance characteristics shall be based on the original result. Comparison with a similar device detecting the same genomic target is insufficient for resolution of discrepant results 4. All results that are indeterminate by the IVD shall be included in the denominator data for analysis 5. All invalid results shall be recorded and evaluated in comparison to the reference result. Invalid results should be reported as individual categories (e.g. internal control failure, extraction failure, etc.) and not aggregated. Invalid results should be analysed separately in the final performance calculations 6. Archived specimens shall be well-characterised and the impact of freezing shall be validated in 3.05.01. 7. Criteria for the selection of specimens shall be explained (e.g. testing of consecutive patients). In addition, any archived specimens used in the study shall be tested in a randomized, blinded manner interspersed with an appropriate number of negative specimens 8. The reference method shall be an RT-PCR assay with high sensitivity, which is preceded by a chemical lysis step followed by solid-phase extraction of nucleic acid (e.g., magnetic bead	
4.02.03b Clinical sensitivity	1. A minimum of 100 prospective positive specimens shall be tested per specimen type for a symptomatic claim. E.g. if OP swabs, NP swabs, nasal swabs are all claimed specimen types, 100 of each must be tested (see note 6) 2. An additional 40 prospective positive specimens shall be tested per specimen type for an asymptomatic claim		

Aspect	Testing requirements	Notes on testing requirements	Source Documents
	3. Reference testing shall be performed on NP swabs for all claimed specimen types except sputum. In the latter case, the same specimen type shall be used for the reference assay 4. If 100 prospective specimens cannot be collected, it is acceptable to supplement with 50 retrospectively collected SARS-CoV-2 positive specimens from individual patients (see note 6)	extraction). The reference method shall be WHO EUL listed, FDA EUA authorized or FDA 510k cleared. (These criteria will be subject to change in future) 9. The reference method must not contain the same primers/probe (sequence) as the assay under evaluation 10. Percent agreement should be calculated in comparison with the reference assay 11. Percent agreement shall be calculated for each specimen type and not for the aggregated data 12. Ct values (cycle threshold) for the assay under evaluation (including internal control results) and the comparator assay must be provided 13. Clinical performance study protocols shall specify how results in the IVD under evaluation and the comparator assay will be compared and how results in the two assays will be statistically determined to be equivalent or not (e.g. Bland Altman analysis) 14. The following basic information shall accompany each specimen: • The specimen type • The specimen collection date • Date of symptom onset. • Clinical diagnosis (if available) • Severity of symptoms (if known) • Product name, manufacturer and product code of the comparator test used • PCR test results (Ct values of SARS-CoV-2 targets and internal control) for the test under evaluation and the reference test 15. All efforts should be made to test positive clinical specimens from • Different clinical sites • Different age groups if possible (children, adults, elderly)	
4.02.03c Clinical specificity	1. At least 500 RT-PCR negative specimens collected from symptomatic individuals (preferably COVID-19 suspected cases) shall be tested.		

Aspect	Testing requirements	Notes on testing requirements	Source Documents
		• Patients exhibiting the range of symptoms, including patients with mild and moderate disease reflecting the typical use cases	

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