



**Instructions and requirements for Emergency Use
Listing (EUL) Submission:
In vitro diagnostics detecting SARS-CoV-2 nucleic acid
or antigen**

Emergency Use Listing of IVDs

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Abbreviations

AgRDT	Antigen Rapid Diagnostic Test
BAL	Bronchoalveolar Lavage
CR	Change request
EUL	Emergency Use Listing procedure
FDA	Food and Drug Administration (USA)
HAMA	Human Anti-Mouse Antibody
HC	Health Canada
IFU	Instructions for Use
IS	International Standard
ISO	International Organization for Standardization
IVD	In vitro diagnostic
LoD	Limit of Detection
NAT	Nucleic Acid Test
NP	Nasopharyngeal
OCR	Optical Character Recognition
OP	Oropharyngeal
PFU	Plaque-forming Units
PHEIC	Public Health Emergency of International Concern
POC	Point of Care
PMS	Post-market surveillance
RDT	Rapid Diagnostic Test
RT-PCR	Reverse Transcription – Polymerase Chain Reaction
TCID	Tissue Culture Infectious Dose
TGA	Therapeutic Goods Administration (Australia)
UN	United Nations
UTM	Universal Transport Medium
VOC	Variants of Concern
VOI	Variants of Interest
VTM	Viral Transport Medium

1 Introduction

The global spread of COVID-19 has dramatically increased the number of suspected cases and the geographic area where SARS-CoV-2 testing is needed to identify infected individuals. In order to do this, in vitro diagnostics (IVDs) of assured quality, safety and performance are required. The World Health Organization (WHO) revised the Emergency Use Listing (EUL) Procedure (previously referred to as the Emergency Use Assessment and Listing Procedure (EUAL)) on 8 January 2020, to be used primarily during a Public Health Emergency of International Concern (PHEIC). The EUL process is based on an essential set of available quality, safety and performance data. The EUL procedure for IVDs to detect SARS-CoV-2 was established 28 February 2020, is intended to expedite the availability of IVDs needed in PHEIC situations and, in that context, to assist interested UN procurement agencies and Member States in determining the acceptability of using specific products for time limited procurement.

The EUL procedure includes the following:

- **Quality Management Systems Review and Plan for Post-Market Surveillance:** review of the manufacturer's Quality Management System documentation and specific manufacturing documents;
- **Product Dossier Review:** assessment of the documentary evidence of safety and performance.
- **Independent laboratory evaluation:** WHO reserves the right to conduct an independent laboratory evaluation of limited scope if it deemed necessary. The study (studies) can either be conducted during the assessment phase and/or post EUL listing.

2 Intended Audience

This document has been prepared to assist manufacturers in correctly compiling the documentary evidence for the purposes of WHO EUL review of IVDs to detect SARS-CoV-2. It describes the required information to support WHO submissions for SARS-CoV-2 antigen rapid diagnostic tests (AgRDT) intended for point-of care (POC) use and for SARS-CoV-2 nucleic acid detection tests. For submissions of AgRDT that are intended for self-testing the manufacturer must also comply with Annex 2 "Requirements for the validation of SARS-CoV-2 AgRDTs intended for self-testing". This document should be used together with WHO document "Emergency Use Listing (EUL) Procedure"¹ for candidate in vitro diagnostics (IVDs) for use in the context of a PHEIC and the document "Invitation to manufacturers of in vitro diagnostics for SARS-CoV-2 to submit an application for emergency use listing by WHO".² Manufacturers³

¹ This document may be accessed through the following website:

<https://extranet.who.int/pqweb/key-resources/documents/emergency-use-listing-procedure-eul>

² This invitation may be accessed through the following website:

<https://extranet.who.int/pqweb/vitro-diagnostics/coronavirus-disease-covid-19-pandemic-%E2%80%94-emergency-use-listing-procedure-eul-open>

³ For the purposes of the EUL, the following definition applies: "**Manufacturer** means any natural or legal person with responsibility for design and/or manufacture of a diagnostic with the intention of making the

who wish to submit the documentary evidence for an IVD should read these documents carefully and fully adopt the guidance therein to compile a successful submission. Rebranded products are outside the scope of EUL assessment and hence not accepted for assessment.

Note: Manufacturers interested in applying for quantitative antigen detection assays are requested to contact WHO as different requirements may apply.

3 The Submission

3.1 Submission clarity

Manufacturers should make every effort to ensure that their product documentary evidence is clear and well-organized (see section 4.2) to help make the WHO review procedure as efficient and timely as possible.

Note: Clarification of specific data requirements will require discussion between the applicant and WHO. Applicants are strongly encouraged to contact WHO as early as possible to discuss specifics of the application.

3.2 Confidentiality

All information submitted in the product dossier is confidential. WHO assessors will treat all information to which they will gain access during the assessment, or otherwise in connection with the discharge of their responsibilities as confidential and proprietary to WHO or parties collaborating with WHO with respect to the SARS-CoV-2 PHEIC.

3.3 EUL submission requirements – Important guidance on documents to be submitted

All items preceded by the symbol “➤” in each section below are required to be submitted as part of the EUL submission. It will be noted in the document where the requirements are applicable to either nucleic acid detection assays, or antigen detection RDTs. If not specified, the requirement is applicable to both types of assays.

The instructions and feedback we provide are subject to change as more is learnt about SARS-CoV-2/COVID-19 and its risk-benefit profile. Any updates will be published on our website as they become available and applicants will be notified.

4 EUL Submission Format

4.1 EUL submission format

- The EUL submission is required to be submitted electronically. Further instruction will be provided to the manufacturer by email when their application is accepted for review.

diagnostic available for use, under his name; whether or not such a diagnostic is designed and/or manufactured by that person himself or on his behalf by another person(s)”.

4.2 Layout and order

WHO requires the following format for the dossier submission:

- Use the format page *1 of 2*, *2 of 2*, etc.
- Clearly divide the submission into sections, as prescribed in this document, and number all pages of each section so that they are uniquely and easily identified.
- Include a table of contents.
- Ensure that all files are identified appropriately. The names should link directly with the sections of the dossier as outlined in this document.
- Font sizes for text and tables are of a style and size (at least font size 12) that are large enough to be easily legible when provided electronically.
- For sections where information is not available, the manufacturer must provide an explanation/justification for not providing the requisite information.

Submissions should be compiled according to the WHO requirements described above. Quality management system documentation should be provided as a separate document to facilitate the efficient review. However, in order to expedite review WHO may accept submissions previously prepared for National Regulatory Authorities if all the information required by WHO is incorporated in such a submission. Manufacturers should contact WHO to determine if a prior regulatory authority submission is appropriate to substitute for the specific sections of the submission.

4.3 Electronic copy requirements

- A searchable PDF is the primary file format used for the electronic copy. However, you must not include any PDF that requires a password to open it.
- The file name should be descriptive of its content and meaningful to the reviewers. The name can be up to 125 characters and can have spaces, dashes (not elongated dashes), underscores, and periods. However, the name of the file must not contain any of the following special characters or it will fail the loading process:
 - tilde (~)
 - vertical bar (|)
 - asterisk (*)
 - forward slash (/)
 - elongated dash (–)
 - colon (:)
 - double quotation marks (“
 - hash sign (#)
 - backward slash (\)
 - apostrophe (')
 - greater than sign (>)
 - single quotation mark (')
 - less than sign (<)
 - various other symbols (e.g., →, *, β, α, ∞, ±, ™)
 - question mark (?)
- All PDF files should be created directly from the source documents whenever feasible (such as sending the document to “print” and selecting to save the print prepared document as a PDF file which should be available in a drop-down menu in the print preview box) rather than creating them by scanning. PDF documents produced by scanning paper documents are far inferior to those produced directly from the source

document, such as a Microsoft Word document and, thus, should be avoided if possible. Scanned documents, particularly tables and graphs, are more difficult to read.

- If submission of a scanned document is unavoidable, we highly recommend that you perform optical character recognition (OCR) so that the text is searchable and clearer. Check to see that the content has been correctly converted by: (1) highlighting an area of text and (2) searching for a word or phrase. If the word or phrase is not returned in the search, then the OCR did not recognize the text. WHO recognizes that use of OCR may not be feasible in some cases for documents with figures and images. Hence, there may be cases in which it is appropriate to have scanned documents in the electronic copy.

4.4 Language and units of measurement

- Submit all documents presented in the dossier in English (unless other arrangements have been made with WHO **prior to** submission of the dossier).
- Any translations of documents must be carried out by a certified translator. Provide an official document attesting to the accuracy of the translation and details on the credentials of the translator.
- All measurements units used must be expressed in the International System of Units (SI) unless otherwise specified.

5 Quality Management System (QMS)

IVDs submitted for the WHO EUL procedure must be manufactured under a suitable, adequate and effective quality management system (QMS).

An assessment of the manufacturer's QMS documentation is a critical step in the reviewing of a SARS-CoV-2 EUL submission. Based on this assessment, WHO decides either to continue with the review of the submission or to request further documentation, or to terminate the application at this point.

The decision to proceed with the review process will be made only if there is sufficient objective evidence that the applicant is the manufacturer, that there is evidence of an adequate QMS in place and that the required manufacturing capacity exists.

The quality management standard *ISO 13485 Medical devices — Quality management systems — Requirements for regulatory purposes* is considered a benchmark in quality management for manufacturers of IVDs by regulatory authorities throughout the world. WHO base their requirements on those identified in this internationally recognized quality management standard.

The following documentation is required to be submitted for review:

- a) Evidence of implementation and maintenance of an adequate QMS, e.g. current ISO 13485:2016 certificate or equivalent, together with the most recent regulatory (or certification body) audit report including audit findings.
- b) A copy of the quality manual including staff organogram.
- c) A list of current quality management procedures.

- d) A copy of the Standard operating procedures for:
 - o Quality control (QC) and batch release procedures;
 - o Design changes;
 - o Control of nonconforming goods/processes;
 - o Supplier evaluation and control, verification of purchased product;
 - o Design and development (including input, outputs, verification and validation);
 - o Complaint handling and vigilance.
 - o Risk management.
- e) The most recent management review minutes.
- f) Manufacturing flowchart including in-process control points.
- g) List of critical suppliers including any outsourced processes with direct product impact (e.g. outsourced manufacturing of components (e.g. conjugated antibodies, strips, reagents), outsourced laboratory testing, packaging, printing, etc.) including details of the supplier for each process and ISO certificates of each of the critical suppliers. If ISO certificates are unavailable, then a copy of the supplier evaluation form.
- h) Name and contact details of the responsible person at the site of manufacture regarding the application.
- i) Full address, including latitude and longitude of the manufacturing facility(s), including warehouse(s) and other facilities used in the manufacturing process.
- j) Site floor plan.
- k) When was the product developed and when was it first placed on the market or the planned timeline for placing on the market.
- l) Provide a list of all countries in which the product under assessment is intended to be marketed. For manufacturers submitting to EUL, it is expected that the product under assessment is intended to be distributed globally, and particularly in low and middle-income countries.
- m) If the product has ever been distributed, please detail the manufacturer's experience with the product (including research-use-only products), especially (but not limited to) number of products distributed, number of customer complaints, if any, type(s) of complaint(s) and customer feedback.
- n) Details on the manufacturing output and capacity (existing inventory, current output, minimum time to provide finished product, maximum batch size, scale up capacity in percentage of current output and required time).

Note: The manufacturer's quality management system must cover all sites currently used to manufacture this product. WHO is required to be notified if any new sites are added.

6 Product Dossier

The product dossier submission should include product descriptive information and documentary evidence of safety and performance. Based on the submitted documentation, a risk-based judgement will be made on whether there is a favorable benefit-risk profile. Applicant are expected to provide the following product information:

6.1 Product information

6.1.1 Regulatory versions

Different regulatory requirements apply to different international markets for IVDs. Manufacturers who market their IVDs to multiple countries often alter some aspects of their products to comply with regional regulatory requirements and marketing needs (e.g. differences in design, information within the instructions for use (IFU), different intended use statements, different batch release procedures, different sites of manufacture, different information on package labels). If such various versions of a product exist, WHO must have a clear understanding of precisely which version of the product the manufacturer is seeking EUL.

- Identify if there are multiple regulatory versions of this product (e.g. provide a table with the different regulatory versions and associated product codes.)
- If the product has multiple regulatory versions, **clearly indicate which regulatory version of the product the manufacturer is submitting for EUL assessment.**
- Ensure that for any of the documents submitted in the product dossier, that the regulatory version to which it relates is identified. Where it is not the version submitted for EUL, a justification for its inclusion in the product dossier should be provided.

6.1.2 Product description including variants (configurations) and accessories

The dossier should include product descriptive information sufficient to allow a dossier reviewer to understand the design applied to the product and how it functions. The IFU may be used to provide some of this information on the condition that it is clearly indicated in the dossier what information can be found in the IFU.

The following information is required to be provided in this section:

- Legal manufacturer.
- Product name and product code(s)/catalogue number(s).
- Overview and intended use of the IVD.

(Note: this may be finalized based on the data and recommendations from WHO).

- Type of IVD (e.g. nucleic acid test (NAT), lateral flow antigen rapid diagnostic test, etc.).
- What the product detects (e.g. qualitative detection of RNA or antigens from SARS-CoV-2).
- The function of the product (e.g. screening, monitoring, diagnostic or aid to diagnosis, staging or aid to staging of disease).
- The clinical indication for the IVD (i.e. specific disorder, condition or risk factor of interest that the product is intended to detect, define or differentiate.)
- Whether the product is automated or manually operated.
- Whether the test is qualitative or quantitative.
- The type of specimen(s) required (e.g. nasopharyngeal/oropharyngeal swabs, nasopharyngeal or nasal wash aspirates, sputum, bronchoalveolar lavage etc.).
- The target population (e.g. symptomatic individuals).
- The intended user (e.g. for NAT, trained laboratory professionals trained in the techniques of real time RT-PCR and IVD procedures, for antigen detection tests, trained healthcare provider, lay user, etc.).

- The intended environment of use (laboratory, point-of-care, near POC, domestic environment etc.).
- A general description of the principle of the assay method or instrument principles of operation.
- For control materials (e.g. positive, negative) to be used or provided with the assay:
 - Include a description of what they are.
 - How they are expected to work (describe their use).
 - Where in the testing process they are used.
 - The concentration of the positive control relative to the limit of detection of the test.
 - How frequently they should be used.
- If a control is commercially available, provide the supplier's name and catalogue number or other identifier.
- A description of the specimen collection and transport materials/medium that are provided with the product or descriptions of specifications recommended for use (e.g. Dacron or polyester flocked swab, with specific brand of transport media validated, as applicable).
- For instruments of automated assays: a description of the appropriate assay characteristics or dedicated assays.
- For automated assays: a description of the appropriate instrumentation characteristics or dedicated instrumentation.
- If applicable, a description of any software to be used with the product.
- If applicable, a description or complete list of the various configurations/variants of product that will be made available.
- If applicable, a description of the accessories, and other products that are intended to be used in combination with the IVD but are not provided.
- If applicable, a description of extraction kits recommended for use with the assay (including the instructions for use).

6.1.3 Testing capabilities

- Briefly describe the current specimen throughput capacity, total time required to perform the test (from clinical specimen collection to result), and number of tests that can be performed per instrument run (if applicable) and per day.

6.1.4 Risk analysis

A risk analysis shall be undertaken to identify and quantify all known or foreseeable hazards⁴ for the product, taking into account such aspects as the user(s) of the device, and the technology involved. The product dossier must contain:

- A summary report of the risks identified during the risk analysis process, including, but not limited to:
 - Risk of false positive and false negative results occurring based on the technology used (e.g. through the reagents used or a high dose hook effect).

⁴ Examples of possible hazards and contributing factors associated with IVDs are given in ISO 14971:2019

- Risk to the patient/community arising from false positive or false negative results.
- Risk of false results based on erroneous use of the product.
- Indirect risks that may result from product-associated hazards, such as instability, which could lead to erroneous results.
- User-related hazards, such as reagents containing infectious agents and chemicals.
- A description of how these risks have been controlled to an acceptable level.
- Measures to inform users of any residual risks.
- A conclusion with evidence that the remaining risks are acceptable when compared to the benefits. This is required to be signed by senior management.
- Evidence that the risk analysis is part of the manufacturer's risk management plan (inclusion of the relevant manufacturer's document).

Note: For AgRDTs intended for self-testing please also refer to Annex 2 (A2.2 Risk analysis).

6.2 Product design and manufacturing information

6.2.1 Product Design

6.2.1.1 Design overview

- Provide information to allow a reviewer to obtain a general understanding of the design applied to the product. A schematic presentation can assist.
- Provide a flowchart of the design process including design inputs and outputs for the product for EUL.

6.2.1.2 Formulation and composition

- For each of the ingredients, provide formulation/composition information.
- If commercial products are used for any of the assay components, provide certificates of analysis, etc.

For NAT:

Please note: Only dual-or triple-target SARS-CoV-2 multiplex assays are eligible for EUL assessment. Single viral target assays are no longer accepted.

- Provide sequences for primers and probes, list of ingredients (including relevant concentrations) for buffers, master mixes, or any other critical components etc.
Note: WHO appreciates that this information might represent proprietary information and assures that all information will be treated strictly confidential.
- Describe the design of the internal control/procedural control, including, but not limited to the type of control (e.g., pseudotype RNA virus), whether they are spiked into each specimen (exogenous) or endogenous (house-keeping gene), the nucleic acid sequence (as appropriate), the sequence of the primer/probes used and its intended function (e.g. NA extraction control, monitor NAT inhibition, reverse transcriptase activity, sample integrity/stability control).

Note: an internal control (IC) must be included in the assay and at a minimum should allow to monitor failure of NA extraction and RT-PCR inhibition. If an endogenous

control (housekeeping gene) is used, the primer/probe design must be specific for mRNA (not genomic DNA).

- If endogenous internal controls are used, provide a schematic presentation of where the primers and probes are located/positioned. If in doubt, contact WHO.

Note: if a housekeeping gene is selected as an internal control, the design of the primer/probes must ensure that only mRNA and not gDNA is detectable (amplified). If the primers and probe target the same exon within a gene, such differentiation is not possible. This applies to a frequently used RNase P internal primer/probe design.

For antigen detection tests:

- Include a detailed description of any capture antibodies and antigens used both for the test and control reactions, how they were designed and purified. For example:
 - Whether monoclonal or polyclonal antibodies used.
 - Whether manufactured in house or purchased commercially.
 - What species they are derived from.
 - What epitope is targeted by the antibodies used in an assay.
 - Provide the components of the conjugates used (antigen or antibody and colour probe) and conjugation method.
 - Indicate if the test uses biotin-streptavidin/avidin chemistry in any of the steps for coupling reagents.

6.2.1.3 Biosafety & biohazard

In this section, the applicant is required to provide evidence demonstrating that correct use of the product is safe; and any information relating to the design, use and disposal of the product that assures safe use under conditions where the product is likely to be used.

- Provide evidence that the following aspects (as applicable) have been considered and means taken to minimize the risk and inform the user of any residual risk:
 - Specimen type.
 - Specimen collection.
 - Specimen processing.
 - Inactivation of specimen (if inactivation is claimed, evidence must be provided).
 - Safe disposal.
- Provide the specific section applicable if reference is made to the submitted risk analysis.
- If reference is made to published biosafety guidelines, include an explanation as to how these have addressed all identified risks relevant to the assay under assessment.

6.2.1.4 Documentation of design changes

- Provide the date of design lock-down.
- Have any design changes been applied to the product? If so, provide records of each design change for the product submitted including the reasons that each change was made.
- Provide references to validation/verification data to support each change.

6.3 Product performance specification and associated validation and verification studies

The manufacturer must submit, where available, evidence of relevant investigations to support the intended use.

a) For each analytical study to be submitted, the following must be provided:

- Study description, study identifier, product code, product identifier (for example, lot numbers), IFU version used, the date of initiation and the date of completion.
- Clearly defined acceptance criteria and an explanation as to how they were derived.
- A summary of the study findings including a conclusion that clarifies how the study objectives have been met.
- The study protocol and full report.
- Analytical studies should be based on the entire testing procedure using the swab supplied with the kit
- Raw data (e.g. file extractions from the PCR instrument, photographs of RDTs or numerical values if a reader is used) may be requested to supplement the study report if required.
- For antigen detection RDTs: provide the colorimetric grading scale used for results interpretation. WHO expects to see graded results during validation, even if a grading scale does not apply to the end user (i.e. as a minimum: non-reactive (0), weak reactive (+), low medium reactive (++) , high medium reactive (+++) , strong reactive (++++)). If a reader is used, numerical values should be provided.
- For all other antigen detection tests provide detailed information about the calibration and interpretation of the results for qualitative or quantitative operation.

- **When studies are still in progress (e.g. shelf life stability studies), the manufacturer must provide the study protocol and study plan along with anticipated dates of completion and submission to WHO.**

b) For clinical studies, the following must be provided:

- Study description, study identifier, product code, product identifier (for example, lot numbers), IFU version used, the date of initiation and the date of completion.
- Clearly defined acceptance criteria and an explanation as to how they were derived.
- A summary of the study findings including a conclusion that clarifies how the study objectives have been met.
- The clinical study protocol(s), including inclusion/exclusion criteria and enrollment strategy.
- The full clinical study report, including raw data (e.g. file extractions from the PCR instrument).

6.3.1 Analytical performance

6.3.1.1 Stability of specimen(s)

This section contains information on the collection, storage and transport of specimens to be used:

- Identify the different specimen types (e.g. nasopharyngeal/oropharyngeal swabs, nasal wash aspirate, sputum, bronchoalveolar lavage) that can be used with the product, including detailed information for each solution claimed in the IFU (e.g. use of different swab transport media (VTM, UTM, saline etc.)).
- The claimed specimen types are expected to align with the current guidelines for laboratory testing (e.g. upper or lower respiratory material).^{5, 6}
- A specimen stability study is required to support the specimen stability claims in the IFU: provide studies/references in support of specimen stability claims for each specimen type.
- If in the clinical study, retrospective clinical specimens that have been frozen are tested, then it is also required to conduct fresh versus frozen studies to support use of these specimens. Where real time studies are conducted:
 - They must include storage conditions (e.g., duration at different temperatures, temperature limits, freeze/thaw cycles), transport conditions and intended use (i.e. the maximum allowable time between specimen collection and its processing or addition to the assay in the setting where testing takes place).
 - A minimum of 10 specimens containing the analyte at a low concentration e.g. 2-3 x LoD (of the NAT or qualitative antigen detection or 2-3 x cut-off dilution for reader-based assays) in the appropriate specimen type and matrix should be tested at each storage condition.
Comment: The “cut-off dilution” of a specimen is the maximum dilution (lowest concentration) that gives a result at or above the cut-off.
 - When the test is intended to be performed on the specimen immediately or shortly after obtaining the specimen, specimen stability testing could be relatively short (i.e. 2 hours at room temperature).

Manufacturers may submit data on later storage time points in agreement with WHO.

6.3.1.2 Validation of specimens – matrix equivalence studies

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 6.3.1.4 Precision, 6.3.1.6 Analytical specificity, 6.3.1.11 Robustness, and 6.3.1.12 Stability of the IVD.

If the manufacturer chooses to test only one representative specimen type or matrix in these analytical studies, the following study is required to be submitted.

⁵ <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance>. This information may change as we learn more about coronavirus disease COVID-2019

⁶ <https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html>

- Where the assay can be used with multiple specimen types (e.g. nasopharyngeal/oropharyngeal swabs, nasopharyngeal or nasal wash aspirate, sputum, bronchoalveolar lavage), a matrix equivalency study should be conducted to establish the relationship between specimen type and IVD performance.
- Similarly, a matrix equivalent study should be done for specimen types collected in different matrices (e.g. use of difference swab types and transport media for nasopharyngeal/oropharyngeal samples, etc.).
- The following conditions should be met in the matrix equivalence study:
 - The study should include a minimum of four positive specimens: one low positive (e.g. approx. 2-3 x LoD or cut-off dilution) and three moderately positives (e.g. approx. 5-7 x LoD or cut-off dilution); and one negative specimen for each claimed specimen type/matrix.
 - Contrived specimens obtained by spiking negative specimens with the appropriate amount of analyte may be used.
 - Test the five specimens in duplicate and compare the results between the matrices.
 - For visually read tests (e.g. antigen detection RDTs), blinding and randomization of the specimens should be included in the experimental design.
- For **antigen detection tests**: for assays validated using positive and negative retrospective specimens in transport media (e.g. UTM, VTM), equivalency with freshly collected and processed positive specimens shall be evaluated (i.e. not stored in transport media). **See section 6.3.2. Clinical evidence for additional requirements.**
- Please note that this study aims to assess potential inhibitory effects of the matrix. LoD, clinical sensitivity and specificity must be performed in **all claimed specimen types**, regardless of any specimen type equivalencies.

6.3.1.3 *Metrological traceability of calibrators and control material values (when reference material is available)*

For NAT:

- All materials used in the validation of the assay must be calibrated against the First WHO International Standard for SARS-CoV-2 RNA NIBSC code: 20/146.
- If other sources of quantitated material has been used, a detailed report from the manufacturer for the *in vitro* transcribed, armored RNA or pseudotyped RNA viruses should be provided (including e.g., the GenBank accession number the sequence is based on), and the relationship to the International Standard (IS) must be established.

For antigen detection tests:

At present, no validated reference materials are available for SARS-CoV-2 assays. Once an IS or reference material has been established, all materials used in the validation of the assay must be calibrated against the established IS or reference material.

- Recombinant proteins may be used as a source of quantitated material.
 - Detailed information on how the protein was expressed and a justification/supporting studies how the recombinant protein resembles the native target protein.
- A detailed report from the manufacturer for recombinant proteins should be provided.

- This material can be used to determine the LoD or sensitivity at cut-off as applicable by testing a dilution series of the recombinant antigen diluted in an appropriate negative specimen (see section 6.3.1.5 Analytical sensitivity).
- Once the LoD or the sensitivity at cut-off has been established, cell culture-derived live virus should be used. Cell culture derived virus should be spiked into a relevant negative specimen and calibrated against the recombinant protein and subsequently used in the other analytical studies.
 - If inactivated virus is used, the inactivation procedure must be explained and equivalency with live virus must be demonstrated.

For NAT & antigen detection tests:

- A detailed report from the supplier of the cell-culture derived virus, including the source, passage history and quantitation (PFU/mL or TCID₅₀/mL) must be provided. Furthermore, the virus stock should be characterized by PCR and the value in copies/ml provided.
- Calibrators and control materials used in the assay should be traceable to a validated reference material for SARS-CoV-2 (e.g. reference material from a National Control Authority, National Standards or the WHO IS).

6.3.1.4 Precision (repeatability and reproducibility)

a) Repeatability

This section includes repeatability estimates and information about the study used to estimate, as appropriate, within-run variability.

b) Reproducibility (intermediate precision)

This section includes information about the study used to estimate, as appropriate, variability between-days, runs, sites, lots, operators and instruments.

- Both repeatability and reproducibility studies should include a minimum of one negative specimen, one low positive specimen (e.g. approx. 2-3 x LoD or cut-off dilution) and 1 moderately positive specimen (e.g. approx. 5-7 x LoD or cut-off dilution).

For antigen detection tests:

- If controls are provided, reproducibility (variability between lots and runs) must be estimated.

For NAT:

- Acceptance criteria should be defined that describes the maximum amount by which the Ct value can deviate before acceptable performance is said to be affected. %CV of below 5% are strongly encouraged.
- The studies can be combined into a single study with an appropriate study design which will allow for robust statistical analysis of repeatability and reproducibility.
- **See Annex 1: Bridging studies for open molecular assays for additional considerations.**

6.3.1.5 Analytical sensitivity (limit of detection (LoD))

- LoD must be determined for each claimed specimen type, irrespective of matrix equivalency.
- The potential impact on emerging variants (at a minimum VOCs) on analytical sensitivity must be evaluated⁷.

For NAT:

- **The LoD of the IVD must be determined utilizing the entire test system from specimen preparation, nucleic acid extraction, to detection.**
- The manufacturer is required to estimate analytical sensitivity using the First WHO International Standard for SARS-CoV-2 RNA NIBSC code: 20/146.
- A tentative LoD can be established through limiting dilutions of the spiked material, followed by nucleic acid extraction, with 3-5 replicate measurements.
- Nucleic acid extraction is required to be performed on each dilution.
- Once a tentative LoD is established, the LoD should be confirmed by testing at least 20 replicate dilutions spanning the tentative LoD to obtain a more accurate estimate of the LoD (to demonstrate that the organism was detected with a minimum 95% positivity (19/20)).
- If this confirmatory study achieves a positivity of 100%, then a lower concentration needs to be tested (with 20 replicates) until <100% positivity is obtained.
- **See Annex 1: Bridging studies for open molecular assays for additional considerations.**

For NAT, list/describe the following in this section (or refer the reviewer to section 6.3.1.3 if the requested information was provided there):

- Titres, lineages and if available NCBI GenBank or GISAID accession numbers of the SARS-CoV-2 stocks used for the LoD study; a description on how the organism stocks were prepared and how the titres were determined.
- The dilution factor and number of serial dilutions of the characterized SARS-CoV-2 specimen that were tested to determine the LoD.
- The nucleic acid extraction/purification method, extraction platform (if applicable) and elution volume, PCR instrument and cycling conditions.

For antigen detection tests:

- The LoD or the sensitivity at cut-off as applicable must be determined **utilizing the entire test system from specimen preparation to detection**. If testing of specimens collected in VTM is claimed, this may result in a dilution factor and should also be evaluated in the analytical sensitivity study as applicable (e.g. specimen with and without the transport media).
- Contrived specimens obtained by spiking clinical matrix collected from SARS-CoV-2 negative individuals with the appropriate amount of analyte should be used.
- In the absence of an IS or reference material for antigen detection assays, it is acceptable to use cell-cultured virus (see 6.3.1.3), diluted in an appropriate negative

⁷ <https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/>

specimen (collected from SARS-CoV-2 negative individuals) to establish the LoD or sensitivity at cut-off.

- Recombinant SARS-CoV-2 protein may be used to establish the LoD or sensitivity at cut-off, if the relationship between live virus and recombinant protein has been established.
- A tentative LoD or sensitivity at cut-off can be established through limiting dilutions of the prepared material with 3-5 replicate measurements.
- Once a tentative LoD or sensitivity at cut-off is established, it should be confirmed by testing at least 20 replicate dilutions (2-fold (log₂) spanning the tentative LoD to obtain a more accurate estimate of the LoD or sensitivity at cut-off (to demonstrate that the antigen was detected with a minimum 95% positivity (19/20)).
- If this confirmatory study achieves a positivity of 100%, then a lower concentration needs to be tested (with 20 replicates) until <100% positivity is obtained.
- In absence of an IS, the LoD and sensitivity at cut-off can be reported in ng/mL, RNA copies/mL, or infectious units. Please note if the LOD is reported in infectious units or RNA copies/mL, the same virus stock preparation must be used for all analytical studies.

For antigen detection tests, list/describe the following in this section (or refer the reviewer to section 6.3.1.3 if the requested information was provided there):

- Titres and lineages of the SARS-CoV-2 stocks used for the LoD or cut-off study; a description on how the organism stocks were prepared and how the titres were determined.
- Furthermore, the virus stock should be characterized by PCR and the value in copies/ml provided.
- The dilution factor and number of serial dilutions of the characterized SARS-CoV-2 specimen that were tested to determine the LoD.

6.3.1.6 *Analytical specificity*

a) **Interfering substances**

Testing of potential interfering substances is required. The evaluation is conducted to demonstrate that the potential interfering substances do not generate false positive results in known negative specimens, and do not lead to false negative results in known positive specimens.

For NAT that use conventional PCR and/or well-established extraction methods prior to testing (e.g., Boom method and column-based extraction methods) interference studies are not necessarily required for respiratory specimens.

For NAT with extraction procedures that are new or for nucleic acid-based technologies that are different from conventional PCR (e.g., various isothermal methods),

- testing of potential interfering substances is required and is dependent on the specimen type.

For antigen detection tests and applicable NATs (see above),
the following information is required:

- List the interfering substances tested and concentrations used.

- Endogenous and exogenous substances should be spiked into the appropriate negative specimen at the highest levels found in individuals.
- Each endogenous and exogenous specimen must be tested unspiked and spiked with the analyte at an appropriate low concentration (e.g. approx. 2-3 x LoD or cut-off dilution).
- Samples should be tested in triplicate and only one claimed specimen type and matrix is required to be included in these studies.
- The tables below indicate the potentially interfering substances that may be found in respiratory specimens.
- If non-respiratory clinical specimen types are claimed, additional substances may need to be considered. Please contact WHO for further information.

See the table below for evaluation of interfering substances for the ability to generate false positive and negative results:

Table 1: Potential interfering substances

Potential Interfering Substance
Respiratory Specimens
Mucin: bovine submaxillary gland, type I-S
Blood (human)
Nasal sprays or drops
Nasal corticosteroids
Nasal gel
Throat lozenges, oral anaesthetic and analgesic
Anti-viral drugs
Antibiotic, nasal ointment
Antibacterial, systemic
Human Anti-mouse Antibody (HAMA)
Biotin

b) Cross reactivity

WHO requires testing of near-neighbour species/strains, of organisms whose infection produces symptoms similar to those observed at the onset of COVID-19, and of the normal or pathogenic microflora that may be present in specimens collected.

Laboratory testing

- Concentrations of 10^6 CFU/ml or higher for bacteria and 10^5 PFU/ml or higher for viruses are recommended. Test specimens can be prepared by spiking cultured isolates into negative clinical matrix.
- Samples should be tested in triplicate and only one claimed specimen type is required in this study.
- Omissions from actual laboratory testing should be supported by a well-documented justification that includes a due diligence attempt to obtain the organisms (and/or purified nucleic acid).

Table 2: Cross-Reactivity: List of Organisms to be tested (and/or analysed *in silico* for NAT)

Other high priority pathogens from the same virus family	Laboratory testing	<i>In silico</i> analysis (for NAT)
Human coronavirus 229E	✓	✓
Human coronavirus OC43	✓	✓
Human coronavirus HKU1	✓	✓
Human coronavirus NL63	✓	✓
SARS-coronavirus	✓	✓
MERS-coronavirus	✓	✓
Other high priority organisms		
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	✓	✓
<i>Chlamydia pneumoniae</i>	✓	✓
<i>Haemophilus influenzae</i>	✓	✓
<i>Legionella pneumophila</i>	✓	✓
<i>Mycobacterium tuberculosis</i>	✓	✓
<i>Streptococcus pneumoniae</i>	✓	✓
<i>Streptococcus pyogenes</i>	✓	✓
<i>Bordetella pertussis</i>	✓	✓
<i>Mycoplasma pneumoniae</i>	✓	✓
<i>Pneumocystis jirovecii</i> (PJP)	✓	✓
Pooled human nasal wash - <i>to represent diverse microbial flora in the human respiratory tract</i>	✓	✗
<i>Candida albicans</i>	✗	✓
<i>Pseudomonas aeruginosa</i>	✗	✓
<i>Staphylococcus epidermis</i>	✗	✓
<i>Streptococcus salivarius</i>	✗	✓

***In silico* analysis for NAT**

- The analysis should include multiple representative strains from GenBank sequence database⁸ 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

⁸ <https://www.ncbi.nlm.nih.gov/genbank/>

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.

- 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.
- If non-respiratory clinical specimen types are claimed for diagnostic use with your device additional organisms may need to be considered.

c) **Microbial Interference Studies**

Microbial interference studies aim at demonstrating that false negatives for SARS-CoV-2 will not occur in presence of other microorganisms.

For NAT:

If *in silico* analysis reveals $\geq 80\%$ homology between the microorganisms 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 studies should be considered:
 - 1) a microbial interference study with SARS-CoV-2 and the microorganisms that the test primers/ probe(s) have homology to,
 - 2) as an alternative to the microbial interference study, you may provide justification as to why (e.g. amount of primer(s)/ probe(s) included in your master mix) the performance of your test would not be impacted by the presence of a causative agent of a clinically significant co-infection, or
 - 3) explain why the *in-silico* results are clinically irrelevant.
- In the case of the microbial interference study, interference should be evaluated using samples spiked at a low (2-3 x LoD) SARS-CoV-2 concentration and a high interferent level (either microorganisms or nucleic acids purified from them), to represent the worst-case scenario, with a minimum of 3 replicates.
- 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.

For antigen detection tests:

- Please provide a list of common pathogens or commensal organisms found in the claimed specimen types.
- Prepare contrived specimens with SARS-CoV-2 and common organisms found in that specimen type and conduct a microbial interference study.

In silico analysis for the prediction of cross-reactivity is less reliable for proteins than for nucleic acid, however, if a sufficient explanation is provided, addressing e.g. the impact of post-translational modifications, conformational vs linear epitopes, such data might be used to support exclusion of microorganisms from this study.

6.3.1.7 *Validation of the primer and probe choice*

For NAT:

Evidence supporting the choice of primers and probes sequences must be provided and must include:

- The target gene(s) and sequence for primers and probes.
- The rationale for selection of primers and probes and specific sequences used.
- 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.
- The potential impact of genetic variations with focus on mutations or deletions associated with variants of concern (VOC) and variants of interests (VOI) must be assessed.

6.3.1.8 *Validation of the cut-off value for antigen tests which a numerical output*

For antigen detection tests:

This section provides information on how the assay reader cut-off was established. Provide the relevant studies and rationale for the chosen cut-off, including:

- Analytical data with the description of the study design, including methods for determining the cut-off.
- The population(s) studied (demographics/selection/inclusion and exclusion criteria/number of individuals included/excluded).
- The method and mode of characterization of specimens.
- The statistical methods (e.g. Receiver Operator Characteristics (ROC)) to generate results).

6.3.1.9 *High dose hook effect*

For antigen detection tests:

- If the potential risk of a false negative result has been identified (see section 6.1.4 on risk assessment) the respective analytical study must be provided e.g. using dilution experiments of relevant (high concentration) patient specimens or spiking negative patient specimen with high concentration analyte.
- The mitigation steps taken by the manufacturer should be described.
- A remaining risk of false negative results identified due to a high dose hook effect must be described in the IFU.
- If the manufacturer does not consider that the assay has a potential for high dose hook effect, the rationale to support this must be provided.

6.3.1.10 *Validation of assay procedure*

For NAT: Procedural control

- If an endogenous internal control (housekeeping gene) is used as part of the assay design, an acceptable range of Ct values needs to be determined for each specimen type.
- If an exogenous internal control is used as part of the assay, evidence for the acceptable Ct range must be provided.

For antigen detection: Quality control accessories and within-device procedural control band

- The product must at a minimum include a procedural control band indicating that sufficient flow has occurred (RDT). Furthermore, it is recommended that the IFU includes instructions to achieve reasonable quality control.
- **For immunoassays (not RDTs)**, please indicate what failure modes the control indicates (e.g. insufficient volumes used, incorrect timing, instability of the system, incorrect specimen used) and provide the corresponding evidence.

6.3.1.11 Flex and robustness studies

This section provides information to demonstrate that the product design is robust, e.g., insensitive to environmental and usage variation. Robustness (flex) studies are designed to challenge the system under conditions of stress to identify potential device deficiencies, including failures, and determine the robustness of the product.

For NAT:

- The influence of the following factors on expected results (both positive and negative) should be considered as applicable:
 - Specimen and/or reagent volume.
 - Handling contamination (e.g. from latex, powder, hand lotion, sweat, and/or soap, etc. as appropriate).
 - Operating temperature.
- The robustness of the instrumentation that is part of the IVD (both extraction and amplification) should be considered.
- For instrumentation that has already been assessed in the context of a WHO prequalified IVD, the data generated as part of the prequalification application may be used to support the EUL application.
- For new instrumentation, the following should be considered:
 - Ruggedness (including the effect of vibration from other instruments).
 - Impact of dust and mold on componentry (e.g. optics).
 - Impact of power/voltage fluctuation.
- Studies investigating the impact of specimen volume should ideally be conducted **in all claimed specimen types**.
- For all other flex studies, **the most common specimen type used for the clinical studies** is required to be tested.
- The test panel should include one negative specimen and one low positive specimen (approx. 2-3 x LoD).
- **Provide a summary of the evidence collected to date and a plan for further testing if such studies are not complete.**

WHO acknowledges that not all studies are applicable or will have been completed when submitting to EUL. However, at a minimum, the effects of sample and/or reagent volumes should be completed prior to submission.

For antigen detection tests:

- The influence of the following factors on expected results (both positive and negative) must be considered.
 - Specimen and/or reagent volume (e.g. including number of drops if applicable).
 - Operating temperature (i.e. incubation temperature) and humidity.
 - Reading times and illumination (visual readings).
- The robustness of the instrumentation that is part of the IVD should be considered relating to
 - Ruggedness (including the effect of vibration from other instruments).
 - Impact of dust and mold on componentry (e.g. optics).
 - Impact of power/voltage fluctuation.
- The most common specimen type used for the clinical studies should be tested.
- The test panel should include one negative specimen and one low reactive specimen (approx. 2-3 x LoD or cut-off dilution).
- **Provide a summary of the evidence collected to date and a plan for further testing if such studies are not complete.**

Note: For AgRDTs intended for **self-testing** the impact of additional conditions on the test result must be considered:

- The influence of mixing the swab in elution buffer (or other reagents): all extremes from not-mixing to vigorous shaking, including generating bubbles and intermediate mixing (i.e. swirling 1 or 2 times) should be addressed.
- Variations (delay/disturbance) in operational steps, e.g. extraction procedure (time of swab in extraction buffer and/or number of rotations of swab in extraction buffer).
- Placement of the test device on non-level surface.
- The impact of different light sources on the visual reading of the control and test lines.
- The effect of moving the test device while it is running (e.g. relocating to another surface or dropping it)
- For AgRDTs that require associated instrumentation or mobile application: the effect of unplugging the device or receiving a phone call while the mobile app is running.

WHO acknowledges that not all studies are applicable or will have been completed when submitting to EUL. However, at a minimum, the effects of sample and/or reagent volumes, operating temperature, and reading times should be completed prior to submission.

6.3.1.12 Stability of the IVD

Shelf-life, in-use stability and shipping stability information provided under this section must be consistent with the instructions for use and product labels provided within the submission.

Note: AgRDTs intended for self-testing may necessitate changes in the packaging, including changes to volumes of liquids, the printing on the outer and inner packaging, and the number of devices and hence to the size of the individual primary and secondary packaging. In these cases, the effect on stability and transport ruggedness must be assessed.

a) **Shelf-life of the IVD including shipping stability**

Accelerated studies or extrapolated data from real-time data are acceptable for initial shelf life claim provided sufficient evidence is provided to support the claim, however, it is a requirement that real time stability studies will be finalized.

Stability studies must be evaluated for the shelf life of reagents. All kit configurations should be tested (or provide a rationale if not). The reagents must be subjected to real or simulated shipping conditions prior to placing them into the shelf-life studies. The following conditions should be investigated (that reflect the environmental conditions of the countries of supply):

- Conditions to mimic extremes of conditions (temperature, humidity, pressure) exposed to during transport/shipping.
- Minimum and maximum storage temperature and humidity range.

Note: WHO acknowledges that not all studies will have been completed when submitting to EUL assessment.

- **In this case, in addition to the study protocol, a plan for the completion of the studies must be provided.**

For NAT:

- Testing of at least one negative specimen, one low positive specimen. Note: Ct values of each replicate, condition and time point must be provided.

For antigen detection tests:

- Testing of at least one negative specimen, one low reactive specimen (2-3 x LoD or cut-off dilution) and ten negative specimens after manufacturing (at T0) and end of the claimed shelf life.
- Testing in triplicate.

b) **In-use stability**

- Provide a report on in-use stability (open pack or open vial stability).
- All labile components (e.g. buffers vials, sealed cartridges, control materials etc.) must be evaluated.
- On-board stability must be tested for an IVD used with an instrument.
- Consideration should be given to operating temperature, humidity range and allowable freeze-thaw cycles of reagents/controls, as applicable.

If a manufacturer utilizes the same instrumentation platform, buffer composition and chemistry as in a WHO prequalified IVD, provide the reference to the prequalified IVD; WHO will give due consideration to leveraging available data which was already assessed.

6.3.2 Clinical evidence

Clinical evaluation is the assessment and analysis of data generated from the clinical intended use of the product in order to verify the clinical safety and performance of the device. Clinical evidence is the combined information from the clinical data and its evaluation. A manufacturer must have clinical evidence to support any clinical claims.

- Specimens from all sections of the population for which claims are made in the IFU must be tested.
- If the intended use includes a claim for testing of asymptomatic individuals, this population is required to be considered in the clinical study.
- The test should be performed by the claimed intended user in the intended testing setting.
- The clinical performance should be evaluated for **each claimed specimen type** (see footnote for exception).⁹
Note: Clinical studies supporting additional specimen types can be submitted at a later time point as a change request.
- Small sample sizes are vulnerable to selection bias. Criteria for the selection of specimens are required to be explained (e.g. testing of consecutive patients). In addition, archived specimens (retrospective testing) should be randomised and tested in a blinded fashion.

Note: Please refer to 6.3. b) for required documentation.

a) Comparator method

For NAT:

- Percent agreement should be calculated in comparison to a comparator assay: a U.S Food and Drug Administration Emergency Use Authorization (FDA EUA) PCR test¹⁰ or a WHO EUL listed PCR test.
- A RT-PCR test with high sensitivity, which is preceded by a chemical lysis step followed by solid-phase extraction of nucleic acid (e.g., magnetic bead extraction), should be used as the comparator method.
- The **comparator assay** must not contain the same primers/probe (sequence) as the assay under evaluation.
- Ct values (cycle threshold) for the assay under evaluation (including internal control results) and the comparator assay must be provided.

For antigen detection tests:

- Percent agreement should be calculated in comparison to a **comparator assay**: a U.S. Food and Drug Administration Emergency Use Authorization (FDA EUA) RT-PCR test or a WHO EUL listed RT-PCR test.

⁹ If nasopharyngeal and oropharyngeal swabs are claimed, it is acceptable for testing to have been conducted in only one of these specimen types.

¹⁰ <https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#2019-ncov>

- A RT-PCR test with high sensitivity, which is preceded by a chemical lysis step followed by solid-phase extraction of nucleic acid (e.g., magnetic bead extraction), should be used as the comparator method.
- Ct values for comparator assay are required to be provided.
- Please note that the clinical performance evaluation requires prospectively collected specimens according to the IFU. If retrospectively collected specimens in VTM are used to complement the dataset, the impact of VTM and storage needs to be understood (see section 6.3.1.3 Matrix equivalency & 6.3.1.5 Analytical sensitivity).

b) Clinical / diagnostic sensitivity

For NAT & antigen detection tests:

- 100 prospective positive specimens should be tested per specimen type.
- If 100 prospective specimens cannot be collected, it is acceptable to supplement with 50 retrospectively collected SARS-CoV-2 positive specimens from patients.

Table 3: Specimens to be tested for a symptomatic claim

Specimen type	No. of specimens from individual patients to be tested for a symptomatic claim	Specimen type for the comparator test*	Comments
Oropharyngeal/ Nasopharyngeal swabs	Combined total of 100	OP/NP swabs	-
BAL/Sputum	Combined total of 100	BAL/Sputum	-
Nasal swabs	100	OP/NP swabs	Please contact WHO for additional information
Saliva	100	OP/NP swabs	
Buccal/oral swab	100	OP/NP swabs	

*If other specimen types are used, this must be discussed with WHO in advance of submission.

For antigen detection tests:

- At least 20% of specimens should have Ct values > 30 on the comparator PCR assay.
- Specimens should be taken at different time points (e.g. days 0-3 (40%); days 4-7 (40%); days >7 (20%)) post-onset of symptoms to understand the dynamics of antigen shedding in respiratory specimens.
- Where the intended use includes a claim for use on asymptomatic individuals, a minimum of 40 asymptomatic subjects (for each claimed specimen type) testing positive for SARS-CoV-2 via comparator method.

For NAT & antigen detection tests:

- The following basic information should accompany each specimen:
 - The specimen type.
 - The specimen collection date.
 - Date of onset of symptoms.
 - Clinical diagnosis (if available).
 - Severity of symptoms (if known).

- Tests used to identify COVID-19 patients.
- PCR test results (Ct values of SARS-CoV-2 targets and internal control).
- All efforts should be made to test positive clinical specimens from
 - Different clinical sites.
 - Different age groups if possible (children, adults, elderly).
 - Patients exhibiting the range of symptoms, including patients with mild and moderate disease reflecting the typical use cases.
- For antigen detection tests, please specify the VTM type (and volume) in case retrospective specimens have been tested, as applicable.

c) **Clinical / diagnostic specificity**

For NAT:

- A minimum of 100 RT-PCR for SARS-CoV-2 negative specimens, collected from symptomatic individuals (preferably COVID-19 suspected cases) are required to be tested.

For antigen detection tests:

- A minimum of 400 SARS-CoV-2 negative specimens (confirmed by RT-PCR), collected from symptomatic individuals (preferably with respiratory symptoms) collected from the general population are required to be tested. Testing should include all claimed specimen types.

d) **Alternative specimen types**

If the manufacturer considers including a claim for alternative specimens (e.g., saliva, buccal/oral swabs, etc.), please contact WHO in advance.

7 Plan for Post-Market Surveillance

Post-market surveillance, including monitoring all customer feedback, detecting and acting on adverse events, product problems, non-conforming goods and processes is a critical component of minimizing potential harm of an IVD listed for emergency use. Certain adverse events should be reported to regulatory authorities in the relevant jurisdiction(s). In the public health emergency settings this EUL procedure serves, it cannot be assumed there are sufficient resources in place to support consistent and effective post-market surveillance but manufacturers must make all efforts possible.

The manufacturer is required to ensure that should the EUL be granted, activities are in place to monitor product safety, quality and performance post-EUL. It is expected that the manufacturer monitors the emergence of variants and assesses the potential impact on product performance.¹¹ It is expected that post-market surveillance activities will be in

¹¹ WHO Information Notice for IVD Users 2021/01 <https://www.who.int/news/item/19-01-2021-who-information-notice-for-ivd-users-2021-01>

accordance with WHO guidance “Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics”.¹²

8 Labelling

The submission must contain a complete set of labelling associated with the product. This includes labels and Instructions for Use (IFU) as well as instrument manual (if applicable) and other instructional materials provided to the user.

Note: For SARS-CoV-2 AgRDTs intended for self-testing please refer to Annex 2 (A2.3) for additional considerations and requirements.

8.1 Labels

- Include high quality copies of all packaging labels for the assay. This includes:
 - Outer labels (secondary packaging).
 - Component labels.
 - (if components are provided without labels, provide information in section 6.1.2).
- These labels must minimally include the following information
 - The product name and product identification number (product code/catalogue number).
 - The name and contact details of the manufacturer, or an authorized representative of the manufacturer, on the outer package labels.
 - The name of the reagent/ingredient.
 - The expiry date.
 - An indication of any special storage and/or handling conditions that apply.
 - The warnings and precautions.
 - The lot/batch and/or serial number.
 - The information regarding particular product conditions such as product sterility.
 - The names of all included reagents in each box on the secondary package label, where possible.
- Where a component is too small to contain all the above information, it must at a minimum contain name, lot number, expiration date, volume, and storage conditions.
- If the product requires associated instrumentation, the above requirements also apply to the instrument.

8.2 Instructions for use (IFU)

The IFU will be reviewed for clarity, correctness, consistency with the information submitted in the dossier, and suitability for the target user group. The following must be submitted in the dossier:

¹² Available on the web page <https://www.who.int/publications/i/item/guidance-for-post-market-surveillance-and-market-surveillance-of-medical-devices-including-in-vitro-diagnostics>

- A copy of the current IFU.
- The instructions for use should comply with the Principles of Labelling for Medical Devices and IVD Medical Devices of IMDRF/GRRP WG/N52 FINAL:2019. Instrument manual

8.3 Instrument manual

- If the product requires associated instrumentation, include a copy of the instrument manual and/or associated operator manuals.

8.4 Any other instructional materials provided to the user

- Provide copies of any other instructional materials that are provided to the user.

9 Contact Information

Any inquiries regarding the EUL should be addressed to: diagnostics@who.int

Annex 1: Bridging studies for open molecular assays

In order to validate performance and to establish equivalent performance of **additional PCR platforms** or **nucleic extraction kits/platforms** the following studies are recommended.

- Testing must be conducted in parallel with the new and original components.

1) Verification of analytical sensitivity (LoD)

- Using one specimen matrix.
- 2-fold (or 3-fold) serial dilution, 3 replicates, until hit rate reaches <100%.
- Confirm LOD with 20 replicates.

2) Precision

- Using the following specimen panel (one specimen matrix):
 - 1 negative specimen.
 - 1 low positive specimen (2-3x LOD).
 - 1 moderately positive specimen (5-7x LOD).

For addition of well-established PCR platforms, commonly used for diagnostic purposes (ABI 7500 (& Fast), Quantstudio 5 (& Fast), Bio-Rad CFX96, Lightcycler 480, Rotor-Gene 6000, Rotor-Gene Q, Stratagene Mx 3005P), the manufacturer should:

- Estimate (at a minimum) variability between runs.

For addition of nucleic acid extraction kits/platforms or any other PCR platforms (not listed above), the manufacturer should:

- Estimate (at a minimum) within-run variability and variability between runs, operators & instruments.

Annex 2: Requirements for the validation of SARS-CoV-2 AgRDTs intended for self-testing

WHO recommends COVID-19 (C19) self-testing as an additional testing approach¹³. The recommendation is based on the use of SARS-CoV-2 AgRDTs intended for self-testing that have equivalent product composition and performance characteristics to AgRDTs for point-of-care (POC) professional use. WHO recommends AgRDTs meet minimum clinical performance requirements of $\geq 80\%$ sensitivity and $\geq 97\%$ specificity when compared to NAT in suspected COVID-19 cases¹⁴.

Manufacturers must recognize that the countries where WHO EUL listed IVDs are procured might present specific challenges related to language, culture, facilities, and circulating variants. IVDs intended for self-testing will need to be designed for this purpose and will require appropriate instructions for use, labelling, robustness, and stability. Changes to the product must be controlled and reported through the usual change notification system.

This annex must be read in conjunction with the entire document. SARS-CoV-2 AgRDTs intended for self/home-testing must be validated as per requirements for AgRDTs for professional POC use (section 6.3 Product performance specification and associated validation and verification studies), Annex 2 only outlines the additional technical requirements for SARS-CoV-2 AgRDTs intended for self-testing. The product dossier must comply with instructions provided in sections 3 (The submission), 4 (EUL Submission Format), 5 (Quality Management System), 6 (Product Dossier), 7 (Plan for Post-Market Surveillance) & 8 (Labelling).

Note: WHO reserves the right to conduct an independent laboratory evaluation of all EUL-listed IVDs or to require manufacturers to participate in blinded testing of their EUL-listed products via a performance panel. The same can also apply to products that are under EUL assessment.

A2.1) Intended Audience

This annex has been prepared to assist manufacturers in correctly compiling the documentary evidence for the purpose of WHO EUL review of SARS-COV-2 AgRDTs that are intended for self- or home-testing¹⁵.

In addition, the product must have been validated for professional POC use as per the requirements outlined in chapter 6.3 in this document: 'Product performance specification and associated validation and verification studies'.

For products that have already undergone WHO EUL assessment for professional POC use or have been assessed and approved/authorized by Health Canada (HC)¹⁶, US Food and Drug

¹³ https://www.who.int/publications/i/item/WHO-2019-nCoV-Ag-RDTs-Self_testing-2022.1

¹⁴ https://cdn.who.int/media/docs/default-source/blue-print/who-rd-blueprint-diagnostics-tpp-final-v1-0-28-09-jc-ppc-final-cmp92616a80172344e4be0edf315b582021.pdf?sfvrsn=e3747f20_1&download=true

¹⁵ In context of this document the phrases self-test and home-test are used interchangeably.

¹⁶ <https://www.canada.ca/en/health-canada/services/drugs-health-products/covid19-industry/medical-devices/testing/home-devices.html#a6>

Administration (FDA)¹⁷ or Australian Therapeutic Goods Administration (TGA)¹⁸ according to published requirements, the following **abridged assessment** strategy (Table A2.1) applies:

Table 4: Abridged assessment pathway

	WHO EUL'd products (professional POC use)	Products with FDA/HC/TGA authorization or approval (professional POC use)	Products with FDA/HC/TGA authorization or approval (self-tests)
QMS assessment (section 5)	yes	Yes	yes
PMS report (last 12 months or since on the market)	yes	Yes	yes
<u>Mini-dossier:</u>			
Product information/design	yes	Yes	yes
Risk assessment	yes	Yes	yes
Product stability for ST configuration	yes	Yes	yes
Flex/robustness*	yes	Yes	yes
Analytical sensitivity	Only for specimen type(s) claimed for ST product	Only for specimen type(s) claimed for ST product	Only for specimen type(s) claimed for ST product
Clinical study (professional use)	Only for specimen type(s) claimed for ST product	Only for specimen type(s) claimed for ST product	Only for specimen type(s) claimed for ST product
Usability study	Yes (Annex 2)	Yes (Annex 2)	Usability study assessed and approved by FDA/HC/TGA**
Labelling review	yes	Yes	yes
Post-listing activities (PMS, CR)	yes	Yes	yes
Independent laboratory evaluation or performance panels	WHO reserves the right to conduct an independent laboratory evaluation of all EUL-listed IVDs or to request the manufacturers to participate in the blinded testing of their EUL-listed products via a performance panel. The same can also apply to products that are under EUL assessment.		

* studies relevant for self-testing (6.3.1.11 Flex and robustness studies)

** Depending on the size of the Usability study performed for HC/FDA/TGA authorization/approval, WHO might ask for additional testing as a post listing commitment.

In order to be eligible/considered for an abridged assessment, the manufacturer must provide WHO with the following information:

- Detailed information on which regulatory versions of the IVD are available and how they differ.

¹⁷ <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas>

¹⁸ <https://www.tga.gov.au/resource/covid-19-rapid-antigen-self-tests>

- Confirmation that the version that has been WHO EUL listed or authorized/ approved by FDA, HC or TGA is the same as the one intended for EUL submission for self/home-testing.
- Inform WHO about any commitments that are in effect with FDA, HC or TGA.
- Inform WHO about any changes made (or planned) to the product (including labelling).

A2.2) Risk analysis (please also refer to 6.1.4)

A risk analysis must be undertaken to identify and quantify all known or foreseeable hazards for the product, taking into account such aspects as the user interface, use of the device, and the technology involved.

- In addition to the 'Risk analysis' requirements described in section 6.1.4, the manufacturer of a self-test must take into consideration at a minimum, but not limited to, the following aspects:
 - Risks resulting in hazardous situations to the individual by sample self-collection (e.g. nasopharyngeal and oropharyngeal).
 - Risks associated with hazardous substances
 - Risks associated with incorrect/inaccurate sample collection
 - Risks resulting in erroneous test results and erroneous interpretation.
 - Risks concerning the disposal of self-tests.
 - Risks concerning the operation & stability of self-tests under the intended environmental conditions (e.g. temperature, humidity, lighting into home environment).
 - Risks concerning identification and management of new and emerging variants of interests (VOIs) and variants of concern (VOCs)¹⁹, including a post-market plan that includes validation using clinical specimens.
- For self-tests utilizing instrumentation and/or software and/or mobile applications, the following aspects must be considered as applicable:
 - Risks concerning accessibility/connectivity issues.
 - Risks concerning operating system(-s), data storage, memory and processor capability.
 - Risks concerning privacy, confidentiality, cybersecurity and legislation in each of the jurisdictions in which the device will be commercialized
 - Risks concerning the complexity of the software systems (including mobile applications).
 - Risks concerning information related to software use, error messages and troubleshooting, etc.
- The summary report of the risks identified during the risk analysis process must include:

¹⁹ <https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/>

- A description of how these risks have been mitigated and controlled to an acceptable level.
- Measures to inform users of any residual risks.
- A conclusion supported by evidence that the remaining risks are acceptable when compared to the benefits. This is required to be signed by senior management.
- Evidence that the risk analysis is part of the manufacturer's risk management plan (inclusion of the relevant manufacturer's document).

A2.3) Requirements for the instructions for use (IFU)

The IFU will be reviewed based on the labelling instructions in chapter 8, section '8.2 Instruction for use'.

Of note:

- Self-tests that do not include printed instructions in their package are not eligible for EUL assessment.
- Instructions for use must be clear and easy to understand; it is strongly recommended that the instruction material includes pictures, quick guides or job aids.
- Quick Guides with images or visual representation that facilitate the performance of the test by a lay user should be provided additionally to the IFU and should be limited to 1-2 pages. The content of the quick guide should agree with the instructions for use and should state clearly the version it relates to.
- IFU must be available in relevant languages (including local languages)²⁰.

Instructions for use of self-tests must contain the following information at a minimum:

- Intended use statement: The language of the intended use statement for self-tests may be simplified, however, key messages must remain (e.g., what the product detects, the function of the product, the required specimen type, the target population, the intended user).
- The clinical sensitivity and specificity of the test (in a self-testing environment) must be clearly identified (including information on the clinical sensitivity/specificity of the test at various time points post symptom onset).
- Information on what variants of SARS-CoV-2 the test can detect, including information of any change in performance due to specific variants.
- Interpretation of results should include pictorial representations of all possible test results (including when a device has failed to provide a valid result).

²⁰ WHO will only review the English version of the IFU, it is the responsibility of the manufacturer to ensure correct translation into other languages.

- Clear information on when testing should be performed, based on clinical performance study results (e.g. test within the first 7 days of symptom onset when viral shedding/viral load is highest).
- Clear warnings on the risk of false-negative results, particularly if testing is not performed within the first 7 days of symptom onset.
- Clear warnings that the tests are less reliable in the later phase of infection and in asymptomatic individuals.
- Clear warning that there is a higher chance of false-negative results with AgRDT self-tests than with laboratory-based molecular tests
- Recommend repeat testing (e.g. within 1-3 days) if ongoing suspicion of infection, high-risk setting or occupational or other requirement.
- Negative results may not mean a person is not infectious and if symptoms are present the person must seek immediate further testing.
- A negative result does not rule out infection with another respiratory pathogen.
- Information on other limitations of the test such as a positive result cannot necessarily determine whether a person is infectious.
- Contraindications (e.g. known cross-reactivity with other pathogens).
- Warnings and Precautions as a result of the risk analysis, robustness or usability studies.
- Storage instructions.
- Guidance on assistance in case it is needed.
- A statement to the user that the test device can only be used once.
- Warnings about the need for supervision in children.
- Information on how to safely dispose of the kit and its contents (waste disposal), including instructions to avoid contamination.
- Instructions on follow-up steps after testing (e.g. the follow-up needed after testing based on results and the national instructions, advice on self-isolation depending on results, where and how to seek professional help, further testing or care etc.).
- Information on residual risks.

For self-tests that utilize instruments and/or software systems (including mobile applications) additional information must be included:

- Clear instructions for lay people on how to use the software, as part of the IFU.
- Minimum technical specifications (memory, operating system, processor capacity etc.).
- Troubleshooting and error resolution.

A2.4) Qualification of usability for self-testing

Overview & purpose of qualification of usability for self-testing

Assessment of product design, labelling and usability of AgRDTs for self-testing should be based on the following studies:

- A) **Labelling comprehension study:** Results of a questionnaire to assess whether the key messages and instructions from packaging and labelling are understood and easily followed by untrained intended users (i.e. self-testers) including understanding the limitations of the assay. The questionnaire should also allow an assessment of whether the user takes the appropriate actions based on a test result according to the IFU (e.g. an understanding of the meaning of negative, positive and invalid results).
- B) **Results interpretation study:** Results of the interpretation of test results study in untrained intended users (i.e. self-testers) of simulated testing (e.g. pre-made and with stable contrived results). Results on devices used in this context must mimic as closely as possible those on the routine device (bandwidths, intensities, colours).
- C) **Observed untrained user study:** Test results and interpretations when the assay is performed by untrained intended users (i.e. self-testers), in an observed setting
- D) **Software Usability studies** (if applicable): Software that is intended to read or analyse results of a SARS-CoV-2 AgRDT should not affect the clinical performance of the test. Usability and user comprehension studies should take into account the ability of the user to interpret the software instructions and ensure that they are clear and easy to follow.

Additional points:

- These studies must only be conducted with instructions and graphics in the languages which will be provided with the commercially available device. It cannot be expected that self-test devices will be used by people with knowledge of international languages and languages need to be adapted according to the setting of use.
- Manufacturers are encouraged to conduct a small-scale human factors study **prior** to starting the final clinical study.
 - These assessment activities will determine the changes needed to optimize the IVD for use by self-testers. Changes may range from minor (simplification of instructions for use without change to the method) to major (e.g. change to the method of specimen collection).
 - Results from any one of the usability studies (A, B, C or D) may indicate that assay redesign is necessary. This may in turn result in a need to revalidate the IVD or to perform additional specific performance studies and to update the risk analysis
- The studies should have pre-defined acceptance criteria and a defined strategy to mitigate risk of errors identified in the study (e.g., modifying the instructions).
- Subjects enrolled in the usability studies must understand that follow-up testing, referral, treatment or care, including isolation, may be needed as per local regulation and guidelines.

Enrollment criteria:

For each of the studies summarized above (A2.4 A-D) the study group must represent a diverse demographic profile of untrained intended users in regards to age, gender, level of education, literacy, geography (urban, rural) and other characteristics that can challenge the usability of the IVD in intended users.

- Subjects should be naive to testing with RDTs; Subjects with prior experience with self-collection or self-testing for SARS-CoV-2 should be excluded.
- Subjects who are trained in laboratory procedures/have experience with laboratory techniques should be excluded.
- Subjects should preferably be from resource-limited settings, with age, gender, level of education, literacy and additional, supplementary skills that can challenge the usability of the IVD
- The range of subjects should include statistically meaningful numbers of all intended users: by e.g. educational level, linguistic ability, economic status, gender, age
- Care should be taken to avoid bias in subject selection. Ideally, the subjects will arrive sequentially at the testing site and be enrolled (after consent) with due regard to the selection criteria. Efforts should be made to avoid convenience sampling of subjects who already know their SARS-CoV-2 status.

A) Labelling comprehension study

- Labelling must be clear and easy to understand; it is strongly recommended that the instruction material includes pictures, quick guides or job aids.
- Additional resources such as a QR code linking to a demonstration video in local language(s) are encouraged. However, this can only be made available during validation studies if it will also be made available to all users of the device when provided commercially
- To assess the ability of users to correctly comprehend key messages from packaging and labelling, questionnaire-based testing of subjects representative of intended users should be performed. The following topics must be addressed:
 - proper self-selection (whether users understand if it is appropriate for them to undertake testing, e.g., whether the test is only for symptomatic vs asymptomatic subjects, whether sequential tests are required etc);
 - understanding key warnings, limitations and/or restrictions;
 - proper test procedure;
 - test result interpretation.
 - understanding how regulations might apply to a test result, including any local regulation, e.g., reporting a positive test result or seeking confirmatory testing
- The questionnaire should be administered to at least 100 subjects, in order to demonstrate comprehension of key messages.

B) Result interpretation study

- This study should cover the ability of users to interpret the correct result: positive, negative, or invalid, and shall consider the extent of lack of agreement between participants as a measure of the clarity of the IFU or the difficulty of interpreting the result on the device
- If the output of the test is from an instrumental interpretation, the participants must be provided with typical contrived items to use in the instrument or, as appropriate, for use on their own smartphones (see also D). Test results read with and without software/instrument/ smartphone must be recorded and analysed.
 - A minimum of 100 subjects should be enrolled to interpret the results of contrived IVDs (for example, static/pre-made tests) to assess their ability to correctly interpret pre-determined test outcomes
 - Subjects can be the same as enrolled for A) Label comprehension study
 - Contrived tests shall be made to demonstrate the following potential test results:
 - non-reactive;
 - range of invalid results including failed control lines
 - reactive;
 - weak reactive (the colour intensity of the line shall be faint and resemble a real clinical test line with reactivity close to that of the assay cut-off (1.5-2 x LoD)).
 - Simulated devices should be static and stable. The colour intensity grading of any test and control lines must not change over time.

C) Observed untrained user study

- The subjects should be enrolled based on the same principle as for studies A) Labelling comprehension study and B) Result interpretation study, however, they should not be the same subjects that participated in those studies.
- The diagnostic sensitivity and specificity (percent agreement) in the hands of the untrained user should be estimated in comparison with the results of the professionally performed RT-PCR test ²¹(approved by HC, FDA, TGA or WHO EUL) on a paired nasopharyngeal or oropharyngeal swab specimen.
- If healthcare providers are collecting the reference sample for comparator testing, it must be ensured that study participants are not provided additional training by observing how healthcare providers collect a sample, particularly if collected from the same anatomical area as the study specimen.

²¹ The test procedure must include a chemical lysis step followed by solid phase extraction of nucleic acid (e.g., magnetic bead extraction)

- Concordance between the subject's self-test result and interpretation of the same result by a trained professional ²²(observer) must also be reported.
- Standard of care protocol at study site and setting must be used to guide referral of participants to further testing or clinical management as needed. Any self-test participant who receives a reactive result should be linked to further testing and care as per standard of care protocol at study site and setting
- Self-testing by at least 90 subjects, i.e. at least 30 who are reactive on the device and at least 60 who are non-reactive, preferably in two geographically diverse populations.
 - Subjects should not be trained or given a self-test demonstration before performing the self-test
 - Each subject should self-collect the test specimen and perform the test according to only those materials provided with the commercial IVD (e.g., instructions for use, labels and other instructional materials with no additional translation or aids).
 - Testing should be performed in a private space with no possibility of interaction with or observation of other subjects.
- Each subject should be observed by a trained laboratory or health care professional during self-testing. The observer should not assist or interact with the subject conducting the test but should note/record errors and other observations during self-testing process.
 - Observation may also be conducted by viewing a video recording of self-testing.
 - Particular attention should be paid to documenting the subjects' compliance with each of the factors raised during risk assessment (ISO 14971) of the process, e.g.
 - Paying attention to the instructions before starting;
 - Correct swabbing technique – insertion position, timing;
 - Correct specimen preparation technique once collected;
 - Application of correct volumes to the IVD;
 - Use of a timing device to use the required times for preparation and reading;
 - Disposal of the specimen collection accessories (e.g. swabs, liquids, extraction tubes);
 - Correct assignment of invalid tests.
 - User techniques and difficulties in operating the system and applying the sample should be documented and reported ("human factors").

²² For WHO purposes, the term "professional use" encompasses a diversity of skills, training and experience, and does not necessarily imply "highest standard of skills, training and experience"

- The observer should also interpret the test result, in a blinded fashion and within the validated reading time stated in the instructions for use to assess results interpretation by subjects.
- Concordance between the subject's self-test result and interpretation of the same results by a trained professional (observer).
- Diagnostic performance (percent agreement) in the hands of the self-user must be estimated in comparison with the results of a professionally performed molecular test (approved by HC, FDA, TGA or WHO EUL) on a paired nasopharyngeal or oropharyngeal swab specimen.

D) Software Usability studies

Software systems might include firmware, mobile applications, cloud systems etc.

- Questionnaire-based testing of subjects (100 lay users) representative of intended users, to assess the ability of such users to correctly comprehend software installation and maintenance activities as well as software erroneous messages. (Can be combined with study A).
- Result interpretation study (see B).
- For Software that is intended to read or analyse results of a SARS-CoV-2 AgRDT, the impact on analytical performance (section 6.3) and clinical performance must be evaluated (see C). Each test result must be read visually (without any software) and in combination with the software.
- Scale-up study (-ies) to assess the data storage capability and interpretation in real-time (especially for cloud-based systems).