Instructions and requirements for Emergency Use Listing (EUL) Submission:

In vitro diagnostics detecting Bundibugyo virus (BDBV) nucleic acid

Emergency Use Listing of IVDs

PQDx_474 version 1.0; 29 May 2026

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Abbreviations

BDBV	Bundibugyo virus
BVD	Bundibugyo virus disease
CN	Cycle number
CR	Change request
Ct	Cycle threshold
EUL	Emergency Use Listing procedure
FDA	Food and Drug Administration (USA)
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
OCR	Optical character recognition
PFU	Plaque-forming units
PHEIC	Public Health Emergency of International Concern
POC	Point of care
PMS	Post-market surveillance
RT-PCR	Reverse transcription – polymerase chain reaction
TCID	Tissue culture infectious dose
TGA	Therapeutic Goods Administration (Australia)
UN	United Nations
UTM	Universal transport medium
VTM	Viral transport medium
WHO	World Health Organization

1 Introduction

On 17 May 2026, the Director-General of the World Health Organization (WHO), after having consulted the States Parties where the event was known to be occurring, determined that the epidemic of Ebola disease caused by *Bundibugyo virus* in the Democratic Republic of the Congo and Uganda constitutes a public health emergency of international concern (PHEIC), but did not meet the criteria of pandemic emergency, as defined in the IHR. The DG statement issued on 17 May 2026 also contained "WHO advice" to States Parties to respond to and prepare for the event.

The World Health Organization (WHO) Emergency Use Listing (EUL) Procedure is primarily used during a PHEIC. The EUL process is based on an essential set of available quality, safety and performance data. The EUL procedure for IVDs to detect Bundibugyo virus (BDBV) nucleic acid is intended to expedite 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 is 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 BDBV nucleic acid, including Ebola and pan-filovirus nucleic acid tests.

This document describes the required information to support WHO submissions for BDBV nucleic acid detection tests, and should be used together with the following WHO documents:

- "Emergency Use Listing (EUL) Procedure" [1]
- "Invitation to manufacturers of in vitro diagnostics for Bundibugyo virus BDBV nucleic acid detection to submit an application for emergency use listing by WHO".
- "Diagnostic testing for Ebola and Marburg virus diseases: interim guidance, 20 December 2024. Geneva: World Health Organization; 2024. https://doi.org/10.2471/B09221[2]".

[1] https://extranet.who.int/prequal/sites/default/files/document_files/EUL-v9August2022.pdf
[2] https://www.who.int/publications/i/item/B09221

Manufacturers[3] 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.

3 The Submission

3.1 Submission clarity

Manufacturers should make every effort to ensure that their product documentation is clear and well-organised (see section 4.2) to facilitate an efficient and timely review.

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 Bundibugyo virus disease (BVD) PHEIC.

3.3 EUL submission requirements – Guidance on documents to be submitted

All items preceded by the symbol "➢" in each section below should be included as part of the EUL submission.

The instructions and feedback provided are subject to change as more is learned about BVD and its risk-benefit profile. Any updates will be published on the PQ IVD website as they become available, and applicants will be notified in due course.

4 EUL Submission Format

4.1 EUL submission format

- ➢ The EUL submission is required to be submitted electronically. Further instructions will be provided to the manufacturer via email once their application is accepted for review.

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. File names must allow the

[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 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)".

reviewer to understand the type of document and the content, in addition to the file number and version. The length of the title should not be longer than 40 characters (e.g. Annex x_6.3.1.5_LOD study v1).

- Font sizes for text and tables are large enough (at least 10-point font) 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.

Quality management system documentation should be provided as a separate document to facilitate efficient review. 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 40 characters and may include spaces, dashes (not en 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 rather than creating them by scanning, PDF documents produced by scanning paper documents are far inferior and more difficult to read than those produced directly from the source document.
- If submitting a scanned document is unavoidable, we strongly recommend performing 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 the 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 measurement 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 review of an EUL submission. Based on this assessment, WHO decides either to continue with the review of the submission, 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 an adequate QMS is in place and that the required manufacturing capacity exists.

ISO 13485:2016 *Medical devices — Quality management systems — Requirements for regulatory purposes* is widely considered the benchmark for quality management by regulatory authorities worldwide. The WHO bases its requirements on this internationally recognized standard.

The following documentation is required to be submitted for review:

If the production site/s is/are certified by MDSAP, please provide the following:

- The latest MDSAP recertification audit report/s, including audit findings (if any) and corrective/preventive actions taken.

If the production site/s is/are <u>not certified by MDSAP</u>, please provide the following:

a) Documented evidence of implementation and maintenance of an adequate QMS (e.g. current ISO 13485:2016 certificate or equivalent, together with the most recent audit report from the regulatory or certification body, including audit findings, if any, and corrective/preventive actions taken).

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 and record forms for:
   - Quality control (QC) and batch release
   - Control of design changes
   - Control of nonconforming products/goods/processes
   - Supplier evaluation and control, verification of purchased products
   - Design and development (including input, outputs, verification and validation)

- Complaint handling and vigilance
- Risk management, including how risks are identified, assessed, and controlled throughout the product lifecycle

e) Minutes from the last two management review meetings.
f) Manufacturing flowchart including in-process control points and the geographical location of each step, especially for multi-site manufacturing.
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/s of manufacture regarding the application.
i) Full address, including latitude and longitude of the manufacturing facility(ies), including warehouse(s) and other facilities used in the manufacturing process.
j) Site/s floor plan.
k) Design lockdown date for the product under assessment and date when it was first placed on the market (or the planned timeline for placing it on the market).
l) List of all countries where the product under assessment is intended to be marketed. For manufacturers submitting to EUL, it is expected that the product under assessment 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) the number of products distributed, the number of customer complaints, if any, the 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. The WHO must be notified if any new sites are added to the manufacturing process.

6 Product Dossier

The product dossier submission should include descriptive information about the product and documentary evidence of its safety and performance. Based on the submitted documentation, a risk-based judgement will be made on whether there is a favourable benefit-risk profile.

Applicants are expected to provide the following information in the product dossier:

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 multiple versions of a product exist, WHO must have a clear understanding of precisely which version of the product the manufacturer is seeking an EUL for.

- 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.

6.1.2 Product description including variants (configurations) and accessories

The dossier must 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 test (e.g., polymerase chain reaction, isothermal amplification, etc.).
- What the product detects (e.g. detection of RNA from BDVD).
- The function of the product (e.g. monitoring, diagnosis of infection or aid to diagnosis).
- 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 readout is qualitative, semiquantitative or quantitative.
- The type of specimen(s) required (e.g., EDTA-whole blood, EDTA-plasma, oral swabs, etc.).
- The target population (e.g. symptomatic individuals).
- The intended user (e.g. for laboratory based molecular tests, laboratory professionals trained in the techniques of real time RT-PCR and IVD procedures, for (near) point-of-care

(POC) molecular tests, trained healthcare professionals, etc.) and the intended environment of use (laboratory, point-of-care, near POC, 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/ID number.
  - 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. sterile Dacron, nylons or polyester 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 hazards[4] 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, carry-over or cross-contamination).

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

- Risk of false results based on genetic mutations of BDBV.
- 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 handling of infectious specimens and 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).

6.2 Product design and manufacturing information

6.2.1 Product Design

6.2.1.1 *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.
- 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, bacteriophage MS2), whether they are spiked into each specimen (exogenous) or endogenous (house-keeping gene), the nucleic acid sequence, the sequence of the primer/probes used and its intended function (e.g., nucleic acid 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 must allow to monitor failure of nucleic acid extraction and RT-PCR inhibition.

6.2.1.2 *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.

- 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.

6.2.1.3 *Documentation of design changes*

- Provide the date of design lock-down[5]
- If design changes have been made after the date of design lock-down, provide records of each design change for the product submitted, including the reasons that each change was made and the supporting validation studies.

6.3 Product performance specification and associated validation and verification studies

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

<u>a) For each analytical study to be submitted, the following must be provided:</u>

- 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.
- A summary of the study findings including a conclusion.
- The study protocol and full report.
- Analytical studies should be based on the entire testing procedure using the specimen collection method specified in the IFU.
- Raw data (e.g., file extractions from the PCR instrument) may be requested to supplement the study report if required.

For any studies that 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.

<u>b) For clinical studies, the following must be provided:</u>

- Study description, study identifier, product code, product identifier (for example, lot numbers), IFU version used, the date of initiation and the date of completion.
- Specimen type and how the specimens were collected and stored if applicable (e.g., dry vs wet swabs and type of media).
- Numeric values (e.g., Ct values, CN values) where applicable, even if the result provided to the end user is qualitative.
- 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.

[5] For WHO prequalification, design lock-down is the date that final documentation, including quality control and quality assurance specifications, is signed off and the finalized method is stated in the IFU.

- The clinical study protocol(s), including inclusion/exclusion criteria and enrollment strategy and/or selection strategy for retrospective specimens.
- The full clinical study report, including raw data (e.g., file extractions from the PCR instrument) and results excluded from the analysis (with a justification).

WHO recognizes the difficulty of obtaining clinical specimens from BDBV infected patients, therefore, preliminary clinical performance data can be generated using contrived specimens spiked into clinical matrix (as describe in section 6.3.2.1). However, the manufacturer shall commit to provide to WHO clinical performance data on prospectively and/or retrospectively collected BDBV positive clinical specimens to maintain the EUL status.

6.3.1 Analytical performance

6.3.1.1 *Stability of specimen(s)*

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

- Identify the different specimen types 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).
  Note: The claimed specimen types are expected to align with the current guidelines for laboratory testing (e.g. EDTA-whole blood, EDTA-plasma, oral and/or buccal swab).
- A specimen stability study report to support the specimen stability claims in the IFU: provide studies/references in support of specimen stability claims for each specimen type.

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

- If quantitated material has been used to validate product performance, a detailed report from the supplier should be provided, e.g., i*n vitro* transcribed, armoured RNA or pseudotyped RNA viruses (including the GenBank accession number the sequence is based on).
  Note: Calibrators and control materials used in the assay should be traceable to a validated reference material (e.g. reference material from a National Control Authority, National Standards or the WHO IS) and/or the reference method used for characterization/ value assignment should be described.

6.3.1.3 *Precision (repeatability and reproducibility)*

This section includes repeatability estimates and information about the study used to estimate, as appropriate, within-run variability. Reproducibility studies used to estimate, as appropriate, variability between-days, runs, sites, lots, operators and instruments should also be submitted.

- Studies should include testing of one negative specimen, one low positive specimen (e.g., approx. 2-3 x LoD) and 1 moderately positive specimen (e.g., approx. 5-7 x LoD).
- Acceptance criteria should be defined that describe the maximum amount by which the output value (e.g. Ct, time to positivity) can deviate before acceptable performance is said to be affected.

Note: 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.

6.3.1.4 *Analytical sensitivity (limit of detection (LoD))*

- **LoD of the IVD must be determined utilizing the entire test system inclusive of specimen preparation, nucleic acid extraction, and detection.**
- LoD should be determined for each claimed specimen type.
- Inactivated virus is the preferred material for estimation of the LoD; however, synthetic RNA can be used as an interim measure.

Note: The manufacturer must commit to determine the LoD using the WHO International Standard if/when it is available.

The following information is requested for LoD studies:

- Titres, lineages and (if available) NCBI GenBank or GISAID accession numbers of the BDBV stocks used for the LoD study; a description on how the organism stocks were prepared and how the titres were determined.
- Detailed step-by step protocol that describes the entire testing procedure.
- The dilution factor and number of serial dilutions of the characterized BDBV 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.
  Note: It is <u>not acceptable</u> to use transport media or extraction buffer without clinical matrix to perform an LoD study.

6.3.1.5 *Analytical specificity*

a) **<u>Interfering substances</u>**

Investigation of potential interfering substances is required to demonstrate that the potential interfering substances do not generate false-positive results in known negative specimens or false-negative results in known positive specimens.

For NATs 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 NATs with extraction procedures that are new or for nucleic acid-based technologies that are different from conventional PCR (e.g., various isothermal methods), the following information is required:

- Refer to Table 1 for the potential interfering substances that should be evaluated.
- Indicate the interfering substances tested and the concentration used for each claimed specimen type.
- 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).
- It is recommended that specimens are tested in triplicate, and only one claimed specimen type and matrix is required to be included in these studies.

Table 1: Potential interfering substances

Potential Interfering Substance	
Whole blood /Plasma Specimens	**Concentration**
Human genomic DNA	
Anti-Nuclear Antibodies	
Rheumatoid factor	
Systemic lupus erythematosus	
Triglycerides	
Haemoglobin	
Unconjugated bilirubin	
Albumin	
Aspirin	
Paracetamol	
Ibuprofen	

b) **Cross reactivity**

WHO requires an *in-silico* analysis of near-neighbour species/strains, of organisms whose infection produces symptoms similar to those observed for BDBV disease, and of the normal or pathogenic microflora that may be present in specimens collected. If laboratory testing has been conducted to investigate potential cross-reactivity, please submit the fully study protocol and report.

In-silico analysis

- The analysis should include multiple representative strains from the GenBank sequence database for each organism (please refer to table 2).
- The full sequence of each organism should be analysed.
- *In-silico* cross-reactivity data should be provided in a tabular form specifying the microorganism, species/strain, accession number and individual % homology of the primers and probes for all targets of your test.
- If *in silico* analysis reveals other potential cross-reactants (i.e. ≥80% homology between one of the primers or the probe to any of the sequences of listed potential cross reactants), carefully review the alignments and determine based on the positions of the homologous stretches and mismatches if additional cross-reactivity and/or interference laboratory testing (refer to microbial interference studies) is required to rule out cross-reactivity or interference of that organism that may affect the performance of your IVD.
    - 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 oral and/or buccal swab clinical specimen types are claimed for diagnostic use with your IVD, additional organisms may need to be considered.

Table 2: Cross-reactivity: List of organisms to be tested (and/or analysed *in silico*)

Other high-priority pathogens from the same virus family[a]	*In silico analysis*	*Laboratory testing – please indicate*
Taï Forest virus	✓	
Reston virus	✓	
Sudan virus - Gulu variant	✓	
Sudan virus - Boniface variant	✓	
Marburg virus - Angola variant	✓	
Marburg virus - Ci67 variant	✓	
Marburg virus – Musoke variant	✓	
Ravn virus	✓	
Orthoebolavirus zairense	✓	
Other high-priority organisms		
Crimean Congo Hemorrhagic Fever virus	✓	
Lassa virus	✓	
Rift Valley Fever virus	✓	
Dengue virus (all serotypes)	✓	
Yellow fever	✓	
West Nile virus	✓	
Japanese encephalitis virus	✓	
Zika virus	✓	
Plasmodium falciparum	✓	
Plasmodium malariae	✓	
Plasmodium vivax	✓	
Plasmodium ovale	✓	
Borrelia recurrentis	✓	
Salmonella typhi	✓	
Coxiella burnetti	✓	
Pseudomonas aeruginosa	✓	
Influenza virus A	✓	
Influenza virus B	✓	
RSV	✓	
Chikungunya	✓	

[a] For IVDs that do not differentiate filoviruses species, viruses from the same family are tested in inclusivity studies.

c) **Microbial Interference Studies**

Microbial interference studies aim at demonstrating that false-negative results for BDBV will not occur in the presence of other microorganisms.

If *in-silico* analysis reveals ≥ 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 are recommended:

- A microbial interference study with BDBV and the microorganisms that the test primers/ probe(s) have homology to.

Note: 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 explain why the *in-silico* results are clinically irrelevant.

- For a microbial interference study, interference should be evaluated using specimens spiked at a low (2-3 x LoD) BDBV 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 tested level, an additional titration study should be performed to determine the highest level of microbial interferent that the BDBV test can tolerate.

d) **Inclusivity Studies**

For tests targeting BDBV, inclusivity must be demonstrated at a minimum using *in silico* analysis for all BDBV strains present in publicly available sequence data banks (e.g., Pathoplexus). Laboratory confirmation of sensitive detection is required if mismatches occur at positions that could impact the performance of the BDBV test kit. Criteria for the maximum number of mismatches and potential positions tolerated by the assay design must be defined, and studies as basis for respective conclusions submitted.
For pan-filovirus NATs, in addition to BDBV strains, inclusivity using in-silico analysis must be demonstrated for all the species claimed in the IFU.

6.3.1.6 *Validation of the primer and probe choice*

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 the selection of primers and probes and the specific sequences used.
- Potential genetic variations, such as mutations or deletions present in individual BVBD strains, need to be evaluated to determine their impact on the assay's detection capability.
- The statistical methods (e.g. Receiver Operator Characteristics (ROC)) to generate results.

6.3.1.7 *Validation of assay procedure*

- 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 through 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.

- 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.

Note: 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 during 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 temperature, condensing humidity, dust and mould on componentry (e.g. optics).
- Impact of power/voltage fluctuation.

- 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 at the time of submission to EUL. However, at a minimum, the effects of specimen and/or reagent volumes should be completed prior to submission.

6.3.1.9 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.

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

Accelerated studies or extrapolated data from real-time data are acceptable for the 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 and submitted to WHO in a timely manner.

Stability studies are requested to support the claimed shelf life of all kit reagents and controls. All kit configurations should be tested (or provide a rationale if not). The reagents should be subjected to 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) that products may be exposed to during transport/shipping.
- Minimum and maximum storage temperature and humidity range.

Note: WHO acknowledges that not all studies will have been completed at the time of submission to the EUL assessment. In this case, provide the study protocol and a timeframe for the completion of the studies.

b) **In-use stability**

- Provide a study 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.

Note: 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

WHO recognizes the difficulty of obtaining clinical specimens from BDBV infected patients, any clinical evidence that the manufacturer has for the product should be submitted for review.

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, including:

- Testing specimens from all sections of the population for which claims are made in the IFU.
- The testing is performed by the claimed intended user in the intended testing setting.
- The clinical performance should be evaluated for **each claimed specimen type.**
- 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, or random selection for archived specimens). 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.

Instructions for use of Comparator method

- Percent agreement should be calculated in comparison to an appropriate comparator assay, such as U.S Food and Drug Administration Emergency Use Authorization (FDA EUA) PCR test or a WHO EUL- listed PCR test.
- An RT-PCR test with high sensitivity, preceded by a chemical lysis step followed by solid-phase nucleic acid extraction (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.
- Numeric values (Ct or CN values) for the assay under evaluation (including internal control results) and the comparator assay must be provided.

6.3.2.1 *Positive and Negative Percent Agreement (PPA and NPA)*

Clinical specimens:

- Provide the number of prospective and/or the number of retrospective positive and negative specimens tested per specimen type.

- Negative specimens shall be collected from symptomatic individuals (preferably BVD suspected cases).
- If archived specimens are used, the manufacturer must describe how specimens were selected and how selection bias was avoided.
- If archived (frozen) specimens are used, the impact of specimen storage must have been investigated as part of "6.3.1.1 Stability of specimen(s)".
- Any archived specimens used in the study shall be tested in a randomized, blinded manner, interspersed with an appropriate number of negative specimens.
- The same comparator test should be used for prospective and archived specimens.
- Ideally, the clinical specimens tested should include at least 20% of weak positive specimens (low virus load) (comparator test results of Ct > 35).
  Note: Contrived weak positive specimens may be prepared by diluting clinical specimens in an appropriate matrix if a sufficient number of natural weak positive specimens are unavailable.

- The following basic information should accompany each clinical 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 BVD patients.
- PCR test results (Ct/CN values of BVDV targets and internal control).
- All efforts should be made to test positive clinical specimens from
  - Different clinical sites.
  - Different age groups, if possible (e.g. children, adults, elderly).
  - Patients exhibiting a range of symptoms reflecting the typical use cases.

Note: If the manufacturer considers including a claim for alternative specimen types (e.g. saliva, urine, 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 different viral species and assesses the potential impact on product performance. It is expected that post-market surveillance activities will be

in accordance with WHO guidance "Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics".[6]

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.

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).

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:

- A copy of the current IFU.

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*

[6] 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

Annex 1: Bridging studies for open molecular assays

To validate performance and to establish equivalent performance of **additional PCR platforms** or **nucleic extraction kits/platforms,** the following studies are recommended at a minimum. Additional studies may be requested for NA extraction kits or PCR platforms that are not well established in the global market or assays intended for point-of-care use.

- 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 the addition of well-established PCR platforms~~,~~ commonly used for diagnostic purposes (i.e., 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 the 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.