

**ANNEX III**  
**INSTRUCTIONS FOR SUBMISSION OF DOCUMENTARY EVIDENCE**

**WHO EVALUATION**  
**OF**  
**MOLECULAR ASSAYS FOR YELLOW FEVER VIRUS**

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## **1. The Submission**

### **1.1 Submitting Documentary Evidence**

This Annex describes the documentary evidence to be submitted in support of a designated Yellow Fever Virus (YFV) molecular assay. This documentary evidence includes evidence to substantiate specific claims of product performance, manufacturability, product stability, suitable instructions as well as the existence of a relevant quality management system.

### **1.2 When to Submit the Documentary Evidence**

Information only needs to be submitted if WHO has favourably reviewed the Expression of Interest, according to the criteria described in Section 4 (“Product Specifications for Entry in the Evaluation Programme”) of the “Invitation to Submit an Expression of Interest for WHO Evaluation of Molecular Assays for Yellow Fever Virus.” When favourable review has occurred, an email will be received outlining the submittal of documentary evidence and other information. WHO reserves the right to ask for information in addition to what is listed herein.

**DO NOT SUBMIT THIS INFORMATION UNTIL YOU HAVE RECEIVED AN EMAIL.**

### **1.3 Submission Clarity**

Manufacturers should make every effort to ensure that their documentary evidence is clear and well-organized to help make the review procedure as effective and efficient as possible.

### **1.4 Submission Requirements – Important guidance on documents to be submitted**

All items in each section below are required to be submitted as part of the submission (or, when indicated, as applicable).

Where information does not exist, (e.g. certain studies), please provide a justification.

**Note: All information submitted as documentary evidence is CONFIDENTIAL.**

## **2. Submission Format**

### **2.1 Submission Format**

Submit one electronic copy of the entire submission.

### **2.2 Layout and Order**

WHO requires the following format for the required documentation:

- Use the format *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 easily identified.
- Include a table of contents.
- Ensure that there are appropriately named file identifiers. The file names should link directly with the sections of the dossier as outlined in this document.

### **Electronic copy requirements**

- PDF is the primary file format used for the electronic copy. However, you must not include any PDF that requires a password in order to open it.
- The electronic copy must be organized as per the format prescribed for the printed copy.

- When creating a PDF from an electronic source document (e.g. Microsoft Word document) avoid using specialist application plug-ins for capture or display data; not all dossier reviewers will necessarily have access to these plug-ins.
- 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 to those produced directly from the source document, such as a Microsoft Word document, and, thus, should be avoided if at all possible. Scanned documents, particularly tables and graphs, are more difficult to read. For any scanned document, we highly recommend that you perform optical character recognition (OCR) so that the text is searchable. Check to see that the content has been correctly converted by: (i) highlighting an area of text and (ii) 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.

### **Language and Units of Measurement**

For the purposes of this evaluation, the following requirements apply:

- 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. Provide both the original and the translated documents.
- All measurements units used must be expressed in the International System of Units (SI).

## **3. Product Information**

### **3.1 Product description including variants (configurations) and accessories**

The submission should include product descriptive information sufficient to allow the reviewer to understand the product and how it functions. The Instructions for Use may be used to provide some of this information. Provide the following information:

- The intended use of the molecular assay (henceforth referred to as a nucleic acid test or NAT)
- What the product detects (the measurand/analyte).
- The function of the product (e.g., screening, monitoring, diagnostic or aid to diagnosis, surveillance).
- Whether the product is automated or manually operated.
- Whether the test is qualitative or quantitative.
- The type of specimen(s) are validated (e.g. serum, plasma, whole blood, etc.).
- The format of the assay.
- The intended testing population (e.g. suspected yellow fever cases, etc.).
- The intended use setting (e.g. laboratory, point of care/testing).
- A general description of the principles of the assay method and instrument principles of operation when applicable.
- Description of all controls (e.g. process and specimen).
- For positive and negative control material to be used with the assay, include a description of what they are, how they are expected to work, and where in the testing process they are 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 that are provided with the product or descriptions of specifications recommended for use.
- For instruments associated with the NAT, a description of these (e.g., readers).
- 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 pack sizes that will be made available.

- A description of the accessories, and other products that are intended to be used in combination with the NAT but are not provided.
- List of d extraction methodologies that have been validated for the assay, when applicable

### **3.2 Product Design - Formulation and Composition**

A description of the components of the assay, and, where appropriate, a description of the reactive ingredients of relevant components (e.g., primers, probes, enzymes).

For instance: Include a description of the primers and probes used in the test, how they were designed (including software), what time frame and data were utilized in the design of the primers and probes what species they are associated with, if commercial products, is there a certificate of analysis, which genomic sequences him been used for in silico specificity analysis, etc.

## **4. Product Performance Specifications and Associated Validation and Verification Studies**

The manufacturer shall submit, where available, evidence of relevant investigations to support the intended use of the product. For each study to be submitted, the following must be provided:

- Study description, study identifier, product identifier (for example, lot numbers), Instructions for Use version used, the date of initiation and the date of completion;
- A summary of the study findings including a conclusion that clarifies how the study objectives have been met; and
- The study protocol and full report. Where the following studies or study reports are not complete or not yet available, the manufacturer shall provide the study protocol and timelines for completion and submission to WHO.

### **4.1 Specimen types/stability/transport**

This section contains information on the types of specimens that have bene validated for use with the assay.

Identify the different specimen types that can be used with the product, including:

- Detailed information for each matrix and anticoagulant, when applicable.
- Provide the studies supporting the use of each specimen/matrix type (and where applicable, anticoagulant).

Provide the studies in support of stability claims, storage claims and, where applicable, claims for transport conditions for each applicable specimen type, including:

- Duration
- Temperatures
- Number of allowable freeze/thaw cycles
- Specimen stability claims

### **4.2 Precision of measurement**

This section describes repeatability and reproducibility studies.

#### **4.2.1 Repeatability**

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

Provide the studies undertaken to establish within-run variability. Such studies should include the use of specimens that represent the full range of expected analyte (measurand) concentrations that can be measured by the product, as claimed by the manufacturer.

#### **4.2.2 Reproducibility**

This requirement contains reproducibility estimates and information about the studies used to estimate,

as appropriate, variability between-days, runs, sites, lots, operators and instruments. Such variability is also known as intermediate precision.

Provide the studies used to establish intermediate precision as appropriate. Include the use of specimens that represent the full range of expected analyte (measurand) concentrations that can be measured by the product, as claimed by the manufacturer.

### 4.3 Analytical Sensitivity

This section includes detailed information about the study design and results to determine the analytical sensitivity of the NAT.

Provide a description of specimen type utilized and preparation including matrix, analyte (measurand) levels, and how levels were established, and the number of replicates tested at each concentration.

If only one strain of yellow fever virus has been used, provide a justification for the strain selected, including reasoning and data to support the use of only one strain.

### 4.4 Analytical specificity

This section describes interference and cross-reactivity studies to determine the analytical specificity, defined as the ability of a measurement procedure to detect or measure only the analyte (measurand) to be detected, in the presence of other substances/agents in the specimen. Note that any related studies should be derived from a comprehensive risk assessment of the test and its intended setting of use. The choice of analytes should be directly linked to the outcomes of such a risk assessment.

#### 4.4.1 Cross-Reactivity

Cross-reactivity and organism interference should be evaluated by testing matrices which are spiked to specific concentrations with pathogens which could potentially cause false positive results and with or without analyte present at 3 to 5x the LoD. Since the availability of NAT positive tested patient sera are very limited alternatively viruses propagated in cell-cultures should be used. To avoid any risk handling highly infectious material the matrices may be inactivated by heat (1h, 60°C) or gamma irradiation (30 kGray). The integrity of matrices treated in either of these manners must be evaluated after the inactivation procedure.

Please provide summary results in the table format as shown below and information related to the methods used to characterize these specimens as positive for the organism utilized. The table does not represent the inclusive list of matrix related cross-reactive organisms which should be tested.

Where possible, use real matrices. If spiked matrices are tested, provide a justification for their use. Provide concentrations at which a pathogen cross-reacts or interferes with the detection of the analyte (measurand). These data should be provided for all specimen matrices claimed in the IFU or justification as to why only one matrix was tested should be provided.

#### Cross-Reactivity: Example table below

Pathogen / Infectious agent*	Number of unique specimens N=	Number of Replicates	Cross-Reactivity (x/6)	Interference (x/6)
Hepatitis C virus	2	3		
Hepatitis E virus	2	3		
Dengue 1 virus	2	3		
Dengue 2 virus	2	3		
Dengue 3 virus	2	3		

Dengue 4 virus	2	3		
Zika virus	2	3		
Tick-borne encephalitis virus	2	3		
Crimean-Congo hemorrhagic fever virus	2	3		
Chikungunya virus	2	3		
Japanese encephalitis virus	2	3		
West Nile virus	2	3		
Lassa virus	2	3		
HIV	2	3		
<i>Plasmodium falciparum</i>	2	3		
Yellow fever 17D vaccine strain	2	3		

\*inactivated virus propagated in cell culture can be used

#### 4.4.2 Interfering Substances

The impact of potentially interfering substances must be evaluated. The evaluation is conducted to demonstrate that the potential interferents do not generate false positive results in known negative specimens, and do not lead to false negative results in known positive specimens. The interfering substances utilized should reflect specimen acquisition and quality (e.g. anticoagulant utilized, hemolysis, lipemia, etc.) and potential therapeutic agents, both over the counter and prescription which could be present due to treatment of symptoms or commonly utilized supplements (e.g. aspirin, acetaminophen, ascorbic acid, etc.).

Please provide summary results in the table format below.

**Interfering Substances: Example table below** for evaluation of interfering substances for the ability to generate **False Positive results (when the analyte is not present)** as well as **False Negative results (when the analyte is present at 3 to 5x the LoD and the interfering substance is also present)**. Note that this example is not an exhaustive list of potential interfering substances that should be tested.

Potential interfering substance	Concentration	False Positive Results (Detected X/3)	False Negative Results (Detected X/3)
Haemoglobin	e.g. >200 g/L or 20 g/dL		
Anticoagulants (specify)			

#### 4.4.3 Inclusivity:

Provide results of testing of all different yellow fever virus strains of African and South American lineage as well as vaccine strains when tested at the limit of detection (LoD). Provide traceability to methods utilized for quantification of the yellow fever virus strains.

Inclusivity: Example table below for evaluation of inclusivity with known strains of yellow fever virus.

Strain Name	Location of Origin	Year of Isolation	Concentration	Results (Detected X/3 at LoD)


#### **4.5 Stability (excluding specimen stability)**

This section describes claimed shelf-life of the NAT for in-use and shipping stabilities. Information provided under this section must be consistent with the Instructions for Use and product labels provided within the submission.

##### **4.5.1 Claimed Shelf Life**

This section provides information on stability testing studies to support the claimed shelf-life. Testing should be undertaken on at least three unique lots manufactured under conditions that are equivalent to and the levels of routine production (these lots do not need to be consecutive lots). The study protocol and reports must specify validity and acceptance criteria and testing intervals. Both accelerated and real-time stability protocols and reports should be submitted. In the case that a protocol has not reach completion and interim report is expected.

##### **4.5.2 In-use Stability**

This section provides information on the in-use stability for the NAT.

Provide the study reports for each assay component (for example, NAT cartridge and buffer).

- For each component, testing is required on a minimum of one lot.
- The studies should reflect actual routine use of the device (real or simulated). This would include open vial stability if a bulk dilution buffer or reagent is utilized in the kit.
- Consideration should be given to multiple access of buffer bottles (opened several times during its use) as well as to different vial size, depending on the presentation in the final kit (e.g. where there may be a 5 mL buffer vial and a 10 mL buffer vial, depending on number of tests), in-use stability must be performed on each vial configuration.
- The study protocol must specify validity and acceptance criteria and testing intervals.
- Both accelerated and real-time stability protocols and reports should be submitted.
- In the case that a protocol has not reach completion and interim report is expected.

##### **4.5.3 Shipping Stability**

This section provides information on shipping stability studies.

- Provide the information and reports generated from studies of at least one lot to evaluate the tolerance of products to anticipated shipping conditions.
- Justifications as the conditions utilized for testing must be provided.
- Shipping studies can be done under real and/or simulated conditions and should include variable shipping conditions such as extreme temperature (heat and/or cold), humidity, light and/or pressure.
- These studies must reflect the environmental conditions of the countries where the product will be and is supplied. The information provided must include a justification for the anticipated conditions.
- The study protocol must specify validity and acceptance criteria and testing intervals.
- If simulated conditions are used, the methods and equipment used must be identified.
- The results and conclusions must clearly demonstrate that the product will met product specifications at the end of its claimed shelf-life after being subjected to the anticipated shipping conditions. As such, it is necessary that after the product has been subjected to the stressed conditions, that there is testing at the end of the claimed shelf life to demonstrate stability.



#### **4.6 Clinical Evidence (Clinical or Diagnostic Sensitivity and Specificity)**

Clinical evaluation is the assessment and analysis of data generated from the clinical intended use of the product 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 and all clinical claims. This must include claims for clinical or diagnostic sensitivity and specificity.

The specimens should ideally be collected from the intended use population and undertaken by user's representative of the intended user group. Manufacturers should attempt to demonstrate performance against different strains, if possible, by using specimens sourced globally. The specimens may be collected prospectively or retrospectively for each claimed type. All attempts should be made to collect natural specimens, but if spiked specimens must be used, this should be noted and justified. In this case, concentrations of spiked material must be recorded, traceability to quantification must be reported along with relevant validation of the procedure utilized, as well as the matrix(es) utilized. Ideally, the base matrix specimens should be sourced from areas where outbreaks of yellow fever are known, or likely, to occur.

#### **Studies demonstrating equivalent performance on certain molecular test platforms**

The following platforms are used in National public health laboratories in Africa. Performance should ideally be demonstrated in at least two of the platforms noted below. A comparison of Ct values can be used to demonstrate equivalence of performance under prescribed run conditions.

- ABI 7500 Real-Time PCR
- ABI 7500 Fast Real-Time PCR
- Qiagen Rotor-Gene Q
- Cepheid SmartCycler
- ABI 2720 Thermal Cycler

### **5. Labelling**

The submission should contain a complete set of labelling associated with the product. This includes

- Labels
- Instructions for Use
- Job aid
- Instrument/application manual (if applicable)
- Additional instructional materials provided to the user

#### **5.1 Labels**

Include copies of all packaging labels for the assay. This includes:

- Outer labels (secondary and tertiary packaging labels)
- Component labels (primary packaging labels)

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 legal manufacturer, or an authorized representative of the manufacturer, on the outer package labels
- The name of the reagent/ingredient
- The expiration date
- An indication of any special storage and/or handling conditions that apply
- Applicable warnings and precautions
- The lot/batch and/or serial number
- The information regarding the particular product conditions such as product sterility
- The names of all included reagents in each box on the outer 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 dedicated instrumentation, the above requirements also apply to the instrument.

## 5.2 Instructions for Use

The Instructions for Use will be reviewed during assessment Stage 3 for clarity, correctness, consistency with the information submitted in the documentation, and suitability for the target user group. The following must be submitted in the documentation:

- A copy of the current Instructions for Use (for the product)
- The instructions for use should comply with the requirements of IMDRF document “Principles of Labelling for Medical Devices and IVD Medical Devices” IMDRF/GRRP WG/N52 FINAL:2019.
- A copy of the job aid

## 5.3 Instrument Manual

If the product requires associated instrumentation (e.g. mobile application), include a copy of the associated manual(s) and/or associated operator manual(s).

## 5.4 Any other instructional materials provided to the user

Provide copies of any other instructional materials that are provided to the user. For instance, if applicable, a copy of the instructions for use of any QC material required but not supplied by the manufacturer.

# 6. Quality Management System

An effective quality management system is a key consideration for all manufacturers of diagnostics. Therefore, NATs submitted for WHO evaluation should be manufactured under an appropriate quality management system. The manufacturer’s quality management system should cover all sites used to manufacture this product.

The quality management standard *ISO 13485:2016 Medical devices — Quality management systems — Requirements for regulatory purposes* is considered to be a benchmark in quality management for manufacturers of NATs by regulatory authorities throughout the world.

WHO bases their requirements on those identified in this internationally recognized quality management standard. Alternatively, an MDSAP certificate is considered equivalent<sup>1</sup>.

Provide evidence of the implementation of a manufacturing quality management system including;

- ISO 13485 certificate or an MDSAP certificate
- Most recent related quality management system (QMS) compliance report
- Copy of the quality manual
- List of valid quality management documentation
- QC and batch release procedure(s)
- Procedure(s) for the control of design and development changes
- Procedure(s) relevant to control of non-conforming goods, including but not limited
- Procedures for corrective and preventative actions, recalls, field safety notices etc.
- Management review reports for the last 12 months
- Detailed diagram of the production workflow including quality control (QC) points (in process and final release activities)
- Critical supplier list including supplied products (components/raw materials) and services

- Details on the experience with the product (when was the product developed and when was it first placed on the market, surveillance and vigilance protocol and updated reports)
- Details on the manufacturing capacity (existing inventory, minimum time to provide finished product, maximum batch size)
- Information on outsourcing or contract manufacturing for any of the components.
- Product and Service reports for the product for the last 12 months including all CAPAs associated with the product over the last 12 months

## **END OF REQUIREMENTS**

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<sup>1</sup> <https://www.fda.gov/medical-devices/cdrh-international-programs/medical-device-single-audit-program-mdsap>