Instructions for Submission Requirements:

In vitro diagnostics (IVDs) Detecting Zika Virus Nucleic Acid or Antigen

Emergency Use Assessment and Listing of IVDs

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1. Introduction

The recent increase in reported cases of microcephaly and Guillain-Barre Syndrome potentially associated with Zika virus (ZIKV) has highlighted the urgent need to identify individuals infected with ZIKV. In order to do this, in vitro diagnostics (IVDs) of assured quality, safety and performance are required. World Health Organization (WHO) expanded the Emergency Use Assessment and Listing (EUAL) Procedure established in July 2015, to ZIKV IVDs in order to determine their eligibility for procurement by WHO and other partners.

The EUAL procedure may include the following sequential steps:

- step 1: review of the manufacturer's Quality Management System documentation;
- step 2: review of the documentary evidence of safety and performance, including labelling and product performance specifications, and associated verification and validation studies;
- step 3: performance evaluation of limited scope to verify critical analytical and clinical performance characteristics.

2. Intended Audience

This document has been prepared to assist manufacturers in correctly compiling the documentary evidence for the purposes of WHO EUAL review of IVDs, and describes the required information to support WHO submissions. This document should be used together with WHO document "Emergency Use Assessment and Listing (EUAL) Procedure for candidate in vitro diagnostics (IVDs) for use in the context of a public health emergency"[1] and the invitation to submit[2]. Manufacturers[3] who wish to submit the documentary evidence for an IVD should read these documents carefully to compile a successful submission.

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[1] This document may be accessed through the following website:
http://www.who.int/medicines/news/EUAL-diagnostics_7July2015_MS.pdf?ua=1

[2] This invitation may be accessed through the following website:
http://www.who.int/diagnostics_laboratory/eual-zika-virus/160211invitation_to_mx_of_Zika_virus_diagnostics_v2.pdf?ua=1

[3] For the purposes of the EUAL, 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)".

3. The Submission

3.1. Submission clarity

Manufacturers should make every effort to ensure that their product documentary evidence is clear and well-organized to help make the WHO review procedure as efficient as possible.
Note: All information submitted in the product dossier is CONFIDENTIAL.

3.2. Submission Requirements – Important guidance on documents to be submitted

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

4. Submission Format

4.1. Submission format

- Submit one printed copy and one electronic copy (exact duplicate of the printed copy in a CD or DVD only) of the entire submission. Submit a signed document attesting that the content of the electronic version is an exact duplicate of the printed copy.
- Provide the printed submission either bound or in a clearly marked set of ring-binders.

***Note*:** **The printed copy will be destroyed by WHO after completion of the review.**

4.2. Layout and order

WHO requires the following format for the dossier submission:

- 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.
- The physical pages of the submission and the page numbers should correspond.
- Ensure that there are appropriately named tab identifiers. The names should link directly with the sections of the dossier as outlined in this document.
- Standard A4 paper is used for all submissions. Text and tables should be prepared using margins that allow the document to be printed on A4 paper. The left hand margin should be sufficiently large that information is not obscured through binding.
- Font sizes for text and tables are of a style and size that are large enough to be easily legible, even after photocopying or when provided electronically. Fonts smaller than 12 points should be avoided whenever possible, except in tables and footnotes where a font size of 10 points is acceptable.

Submissions should be compiled according to the WHO requirements described above. However, WHO may accept submissions previously prepared for National Regulatory Authorities if:

- all the information required by WHO is supplied.

Manufacturers should contact WHO to determine if a particular prior regulatory authority submission is appropriate to substitute for the specific sections of the submission.

4.2.1 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 to open it.
- The electronic copy must be organized as per the format prescribed for the printed copy.
- The name of 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 (“)
  - pound sign (#)
  - backward slash (\)
  - apostrophe (’)
  - greater than sign (>)
  - single quotation mark (‘)
  - less than sign (<)
  - various other symbols (e.g., →,*, β , α ,∞,±, ™)
  - question mark (?)

- When creating a PDF from the source document (e.g. Microsoft Word document), please consider when using Adobe® plug-ins to create PDF files and/or capture or display data, there is a risk that information may not display correctly because reviewers may not have access to certain plug-ins to review content being displayed by a plug-in.

- 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: (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.

4.3. Language and units of measurement

For the purposes of EUAL, 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).

5. Product Information

5.1. 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 IVD (please note: this may be finalized based on the data and recommendations from WHO).
  - What the product detects (the measurand).
  - The function of the product (e.g., screening, monitoring, diagnostic or aid to diagnosis, staging or aid to staging of disease).
  - The 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. serum, plasma, whole blood, etc.).
- The intended testing population (e.g. neonates, antenatal women, symptomatic individuals, etc.).
- The intended user (laboratory professional and/or health care worker at point-of-care).
- The intended setting of use (laboratory, point-of-care).
- A general description of the principle of the assay method or instrument principles of operation.
- For 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 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.

5.2. Product design - Formulation and composition

- For each of the ingredients, provide formulation/composition information. For example, include information such as nucleic acid sequences for primers, ingredient lists for buffers, amino acid sequence details for recombinant proteins, etc.
- Identify the sources of the materials from which the IVD components are constructed.

5.3. Product workflow

- Briefly describe 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 and per day.

6. Product Performance Specification, and Associated Validation and Verification Studies

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

- Study description, study identifier, product identifier (for example, lot numbers), IFU 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;
- The study protocol and full report. Where the following studies are not complete or not yet available, the manufacturer shall provide timelines for completion and submission to WHO.

Note: **When studies are still in progress or plans to commence such studies are in place, the manufacturer should provide an update of progress or the study plan along with anticipated dates of completion.**

6.1. Specimen type

This section contains information on the types of specimens that can be used with the IVD.

- Identify the different specimen types that can be used with the product, including:
  - detailed information for each matrix and anticoagulant, when applicable
- Where the assay can be used with multiple specimen types, please provide a matrix equivalency study for all claimed specimen types and anticoagulants.
  - The matrix in which the clinical studies are conducted is the comparator. All other matrices are to be shown to be equivalent to the comparator matrix.
  - Negative specimens for each claimed specimen type are spiked with the same amount of the analyte and assayed and the results compared.
  - Test contrived specimens consisting of negative, high negative, low positive, and 3-4 values across the dynamic range of your assay.
  - Test five specimens in duplicate for each concentration and compare the results between the matrices.
- Provide the studies/references in support of specimen stability claims, storage claims and, where applicable, claims for transport conditions for each applicable specimen type.

6.2. Precision of measurement

Note: This is an obligatory requirement for IVDs to detect Zika virus antigen. It is an optional requirement for IVDs detecting Zika virus nucleic acid.

This section describes repeatability and reproducibility studies.

6.2.1. 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.

6.2.2. 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.

6.3. Analytical sensitivity

WHO requires each assay to be calibrated/tested against biological reference material when and where available:

- WHO-supplied ZIKV interim International Standard for nucleic acid tests (NATs), estimated availability April – May 2016. This interim standard may be used by assay developers before the final status as an International Standard is attained.
- For those manufacturers who have already submitted to the EUAL, calibration studies with this standard are still required and results need to be submitted to the EUAL as soon as possible.
- For multiplex NATs that detect Chikungunya nucleic acid, a reference standard is available from the Paul Ehrlich Institut, Germany.
- For multiplex NATs that detect other markers e.g. West Nile virus, dengue viruses, reference material is also available from FDA/CBER.

6.4. 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.

6.4.1. Reactivity/Inclusivity

Reactivity is to be evaluated for additional isolates of Zika virus**.**

- Isolates should be tested at or near the limit of detection (LoD) of the assay utilizing the entire test system.
    - Test levels should not exceed 1.5-2 x LoD.
- In addition, reactivity should also be demonstrated by providing sequence alignments using genomic sequences of other Zika virus isolates (including those tested) and the sequences of the assay's primers and probes.

Please provide a summary of the data for each virus strain tested, in each specimen matrix, using the example table below.

Table 1: Example table for reactivity

Zika Virus Isolate*	Source/Specimen Type**	Concentration	Cycle Threshold (Ct)

*Please indicate if this is the same isolate of Zika virus that was used for the LoD studies.
**Please list source of material used and sample type tested (i.e., organism, purified nucleic acid etc.)

6.4.2. Cross-Reactivity

- WHO requires testing of organisms whose infection produces symptoms similar to those observed at the onset of Zika virus infection and also viral strains which have a significant likelihood to result in cross-reactivity due to genetic similarity with Zika virus.

- Organisms/strains which are likely to be observed in the currently affected areas should be tested since these organisms/strains will be an important part of the differential diagnosis of Zika virus infection.

- Consideration should also be given to testing of arboviruses from regions where the vector *Aedes aegypti* is endemic.

- The evaluation should reflect test specimens prepared at the highest clinically relevant level of organism. Test specimens can be prepared by spiking cultured isolates into negative clinical matrix and determining cross-reactivity based on triplicate measurements.

- The following table (Table 2) provides a list of organisms to be included in testing for cross-reactivity.

- 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 – which is acceptable for the purposes of this EUAL).

- If *in silico* analysis of the organisms in Table 1reveals other potential cross-reactants these should also subject to laboratory testing or an explanation should be provided as to why *in silico*-generated data is not clinically relevant (irrelevant isolate, location/extent of match within primer/probe, etc.). In such cases, the results of *in silico* analysis should be included in the review.

Table 2: Organisms to be tested as well as analyzed in silico (for assays detecting Zika Virus) in specimens of intended use

Disease/Infectious agent		% Cross-reactivity	Laboratory testing	In silico analysis
Flavivirus	Dengue virus 1, 2, 3 and 4		✓	✓
	Yellow fever (optional)		✓	✓
	Yellow fever vaccine strain		✓	✓
	West Nile virus		✓	✓
Chikungunya			✓	✓
Parvovirus (B19)			✓	✓
Plasmodium falciparum			✓	✓
Flaviviruses	St. Louis encephalitis virus		✗	✓
	Rocio virus		✗	✓
	Ilheus virus		✗	✓
	Iguape virus		✗	✓
	Tick-borne encephalitis virus		✗	✓
	Japanese encephalitis virus		✗	✓
	Spondweni virus		✗	✓
	Hepatitis C virus		✗	✓
Alphaviruses	(Sindbis virus, Tonate virus and Una virus)		✗	✓
	O'nyong-nyong virus		✗	✓
	Barmah Forest virus		✗	✓
	Ross River virus		✗	✓
	Western Equine Encephalitis virus (WEE)		✗	✓
	Eastern Equine Encephalitis virus (EEE)		✗	✓
	Mayaro Virus		✗	✓
Measles virus			✗	✓
Rubella virus			✗	✓
Adenovirus: all serotypes			✗	✓
Hepatitis B virus			✗	✓
HIV			✗	✓
Varicella Zoster virus			✗	✓
Cytomegalovirus (CMV)			✗	✓
Epstein Barr Virus (EBV)			✗	✓
Rickettsia sp.			✗	✓
Borrelia burgdorferi			✗	✓
Group A *Streptococcus*			✗	✓
Leptospirosis			✗	✓
Plasmodium vivax			✗	✓
Trypanosoma cruzi (Chagas)			✗	✓
Schistosomiasis			✗	✓
Hepatitis A virus vaccine (BIOVAC-A brand)			✗	✓

Salmonella typhi vaccine (Typhoid - Ty21a vaccine)		✗	✓

6.4.3. Interferences – Antigen assays

This section provides information to evaluate the effects of potentially interfering and cross-reacting substances/agents on the assay. The study should include :

- the substance/agent type and concentration tested
- specimen type
- analyte(measurand) test concentration
- a study design that includes appropriate interferents and cross-reacting substances/agents. Typically, interference studies involve adding the potential interferent to the specimen and determining any bias of the test parameter relative to the control specimen to which no interferent has been added. Common interferents and cross-reacting substances/agents include, as appropriate:
  - substances used for patient treatment (e.g. therapeutic drugs, anticoagulants, etc.)
  - substances ingested by the patient (e.g. over the counter medications, alcohol, vitamins, foods, etc.)
  - substances added during specimen preparation (e.g. preservatives, stabilizers)
  - substances encountered in specific specimens types (e.g. haemoglobin, lipids, bilirubin, proteins)

6.5. Limit of detection

The LoD of the IVD should be determined utilizing the entire test system from specimen preparation to detection for each clinical specimen type/matrix claimed. If viral stocks from the currently circulating Zika virus (Brazilian strain) are not available, it is acceptable to use commercially available Zika viral stocks (from previously circulating strains) for the calculation of LoD. The following information provides one means considered acceptable for calculating LoD.

- A tentative LoD can be established through limiting dilutions of the prepared material with replicate measurements in a relevant clinical matrix.

- Once a tentative LoD is established, the LoD should be confirmed by preparing at least 20 additional replicates at the LoD concentration and demonstrating that the organism was detected 95% of the time (19/20).

- If inactivated virus is used in these studies, describe how the material was inactivated and perform a study to determine if the inactivation process impacted the LoD of the IVD.

- For antigen detection IVDs, where recombinant protein is used in these studies, please provide a study demonstrating the relationship/equivalence of the LoD to live and inactivated virus. Once established, the recombinant material can be used in other studies.

***Note 1*:** **Alternative means to calculate LoD may be used.**
Note 2: **Please use the following format when submitting LoD information and data.**

Analytical sensitivity LoD studies determine the lowest detectable concentration of Zika virus at which approximately 95% of all (true positive) replicates test positive. The LoD was determined by limiting dilution studies using characterized samples.

[List the titres and strains of the viral stocks used for the LoD study, and describe how the stocks were prepared and how the titres were determined.]

[**List the dilution factor and number of serial dilutions of the characterized virus/viruses that were tested to identify an end-point for detection with each primer and probe set.]**

[List the nucleic acid extraction/purification method used.]

Serial dilutions of the characterized Zika virus were then tested in **[X number of replicates (three-five recommended)]** replicates. The lowest concentration at which all [**number of replicates**] replicates were positive was treated as the tentative LoD for each test. The LoD of each test was then confirmed by testing **[X number of replicates** (**at least 20 recommended)**] with concentrations at the tentative limit of detection. The final LoD of each test was determined to be the lowest concentration resulting in positive detection of **[X number of positive replicates (at least 19 out of 20 replicates**)].
[**Include analysis of LoD results, indicating the final LoD for each test**.]

Please provide a summary of the data for each virus strain tested, in each specimen matrix, using the example table below.

Table 3: Example table for LoD

Virus Strain Tested	Stock Virus Titre	Serial 10-Fold Dilution Factor	PFU/mL or $TCID_{50}$/mL Dilution	Run 1 C_t	Run 2 C_t	Run 3 C_t	Run 4 C_t	Run 5 C_t	Call Rate	Lowest Concentration with Uniform Positivity per Analyte	LoD per Virus Strain
Example: (analyte)											

- In addition to LoD, extensive inclusivity *in silico* analysis of the assay primer/probes with all known strains (past and present) of Zika virus (including the strain used for LoD) is required.

- For multiplex IVDs, the LoD is required to be calculated and submitted for each virus claimed to be detected.

6.6. Stability (excluding specimen stability)

This section describes claimed shelf life of the IVD, in-use stability and shipping studies.[4] **When studies are not yet completed, please provide a plan for completion of a study.** It is understood that for the purposes of the EUAL, not all studies may be complete. However, it is a requirement that such studies will be finalized.

6.6.1. Claimed shelf life

This section provides information on stability testing studies to support the claimed shelf life.

- Where possible, testing should be undertaken on **at least three different lots** manufactured under conditions that are equivalent to routine production conditions (these lots do not need to be consecutive lots).
- The study protocol must specify acceptance criteria and testing intervals.
- Accelerated studies or extrapolated data from real time data are acceptable for initial shelf life claim **but need to be followed up with real time stability studies**.

[4] 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.

- When accelerated studies have been performed in anticipation of the real time studies, identify the method used for accelerated studies.

6.6.2. In-use stability

This section provides information on the in-use stability for the IVD. Studies should be submitted for each assay component.

- Provide the studies for each assay component (for example, EIA plate, buffer, conjugate, substrate, acid).
- 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 and/or, for automated instruments, on-board stability. Consideration should be given to multiple access of reagent 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 10mL buffer vial, depending on number of tests), in-use stability must be performed on each vial configuration.
- The study protocol must specify acceptance criteria and testing intervals.
- In the case of automated instrumentation, if calibration stability is claimed, then supporting data should be included.

6.6.3. Shipping stability

This section provides information on shipping stability studies.

- Provide the information identified in the introduction to Section 7, from studies of **one lot** to evaluate the tolerance of products to the anticipated shipping conditions.
- 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 of supply. The information provided must include a justification for the anticipated conditions.
- The study protocol must specify acceptance criteria and testing intervals.
- If simulated conditions are used, the methods used must be identified.

6.7. 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 manufacturer must consider multiple skill levels of users, as well as potential instrument and reagent problems. Below is a list of factors that may need to be considered when performing robustness studies:

- Operator error/ human factors, including use of incorrect specimen type, Incorrect application of the specimen to the device (e.g., incorrect placement, incorrect volume), incorrect handling of reagents including those in self-contained unitized test devices, incorrect placement of device (e.g., non-level surface), incorrect placement of reagents, including strips, or other components that contain reagent, use of incorrect reagents (for example, reagents that are not specific for the particular device or lot or generic reagents), incorrect order of reagent application, use of incorrect amount of reagent, incorrect timing of procedures (e.g., specimen application, running the test, or reading results), incorrect reading of test results, incorrect reading due to color blindness, etc.
- Specimen integrity and handling including errors in specimen collection, use of inappropriate anticoagulant, clotted specimens, error in specimen handling, incorrect specimen transport and/or storage, presence of interfering substances, presence of bubbles in the specimen, etc.
- Reagent integrity (Reagent viability) including use of improperly stored reagents, use of outdated reagents, use of improperly mixed reagents, use of contaminated reagents, etc.
- Hardware, software, and electronics integrity including power failure, power fluctuation, incorrect voltage, repeated plugging and unplugging of the device, hardware failure, software failure, electronic failure, physical trauma to unit, etc.
- Stability of calibration and internal controls including factors that affect calibrator and calibration stability, factors that may interfere with calibration.
- Environmental factors including impact of key environmental factors (heat, humidity, barometric pressure changes, altitude (if applicable), sunlight, surface angle, device movement, etc.) on reagents, specimens, and test results, impact of key environmental factors (including changes in parameters such as pH or temperature), etc.

The following should be provided:

- A summary of the evidence **collected to date** that falls within this category and a plan for further testing if such studies are not complete. WHO acknowledges that for a submission to the EUAL, not all studies will have been completed.
- State the test environment and relation to the intended use environment.
- A discussion of what tests were considered for the device and why they were or were not performed.
- If a performance study has been conducted that includes human factors/usability end points, reference to the studies and endpoints should be made, but full results do not need to be repeated.

6.8. 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 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. This will include claims for clinical or diagnostic sensitivity and specificity.

- The performance characteristics of the IVD should be established using a clinical study with a limited number of prospective samples.
- If a prospective study is not feasible, an acceptable alternative would be to test retrospectively collected Zika virus ribonucleic acid (RNA) negative and positive specimens from symptomatic and if possible asymptomatic patients, including pregnant women.
- The study report should include the following information:
    - the specimen collection date
    - date of onset of symptoms
    - tests used to identify Zika virus RNA positive specimens and other cross-reacting virus, negative specimens etc.
    - positive specimens
        - A minimum of 50 positive specimens is required.
        - The number of natural clinical specimens should be at least 25.
        - Contrived specimens can be used to supplement the natural specimens to ensure adequate numbers are tested. These should be made in individual negative clinical matrix obtained from the affected region and collected ideally from febrile patients for symptomatic claims and non-febrile patients for asymptomatic claims, for each clinical matrix claimed for use with the IVD. Half of the contrived samples can be prepared by spiking the Zika virus at LoD and the remaining contrived samples should cover the range of the IVD up to and not higher than 5 x LoD.
        - Manufacturers should attempt to demonstrate performance with different strains, if possible using specimens sourced globally.
        - In order to reduce the level of biosafety required for testing, specimens may be heated (for antigen assays) or lysed (for NAT). Evidence that this does not affect the performance of the test should be documented.
    - Negative specimens
        - a minimum of 100 negative specimens must be tested
        - of these, at least 25 must be sourced from pregnant women (ideally from an affected region)

- Tested must occur in a blinded fashion and percent agreement should be calculated in comparison to a reference/comparator such as bi-directional sequencing.
- When assigning clinical truth for all specimens, optimally specimens obtained from patients with data demonstrating seroconversion should be used i.e. from patients with a pattern of only nucleic acid detectable for early bleeds with results of serial bleeds demonstrating the rise of both Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies.

7. Labelling

Where possible, the submission should contain a complete set of labelling associated with the product. This includes:

- labels
- instructions for use (IFU)
- if applicable, the instrument manual
- any other instructional materials provided to the user

7.1. Labels

- Include copies of all packaging labels for the assay. This includes:
  - outer labels
  - component 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 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 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 instrumentation, the above requirements also apply to the instrument.
- The instrument should clearly display information regarding its status as a new or reprocessed product.

7.2. Instructions for use

The instructions for use 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 instructions for use
- The instructions for use should include, where possible, comply with the requirements of GHTF/SG1/N70:2011 Label and Instructions for Use for Medical Devices.

7.3. Instrument manual

- If the product requires associated instrumentation, include a copy of the instrument manual and/or associated operator manuals. If the instrument manual is large, an electronic version (CD or DVD) may be included instead of a hard copy.

7.4. Any other instructional materials provided to the user

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

8. Quality Management System

An effective quality management system (QMS) is a key consideration for all manufacturers of diagnostics. Therefore, IVDs submitted for the WHO EUAL procedure 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 13485:2003 Medical devices — Quality management systems — Requirements for regulatory purposes* is considered to be a benchmark in quality management for manufacturers of IVDs by regulatory authorities throughout the world. WHO bases their requirements on those identified in this internationally recognized quality management standard.
Provide evidence of the implementation of a manufacturing quality management system including;

- ISO 13485:2003 certificate;
- the most recent QMS audit report;
- a copy of the quality manual;

- a list of valid quality management documentation;
- quality control (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 to procedures for corrective and preventative actions, recalls, field safety notices etc.;
- a recent management review report;
- details of the production workflow including 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);
- details on the manufacturing capacity (existing inventory, minimum time to provide finished product, maximum batch size);
- information on outsourcing or contact manufacturing for any of the components.

9. Contact Information

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