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THE UNITED REPUBLIC OF TANZANIA

MINISTRY OF HEALTH

TANZANIA MEDICINES AND MEDICAL DEVICES AUTHORITY

PROTOCOL FOR PERFORMANCE LABORATORY EVALUATION OF HUMAN IMMUNIDEFICIENCY VIRUS (HIV) SEROLOGY ASSAYS

FIRST EDITION

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ACKNOWLEDGEMENTS

This first edition of the protocol intends to establish a well-documented procedure for conducting performance evaluation of in vitro diagnostic tests for HIV antibody and/or antigen detection. This protocol serves as a guide and set a clear expectation on performance evaluation reports that are mandatory in marketing authorization applications and reduce round of request for additional data which used to prolong the evaluation time and hence delays in approval. It is my hope that the information included in this protocol will assist manufactures and/or applicants to conduct performance evaluation studies in Tanzania Mainland and subsequently expedite verification process of the results by the Authority.

With this, I would like to extend my sincere gratitude to the TMDA staff and all other experts who contributed their valuable time and experience to the development of these guidelines.

Specific acknowledgements are extended to Ms. Rehema Mariki, Dr. Goodluck Gotora, Mr. Christian Kapinga, Ms. Edina Zebedayo, Mr. Edinanth Gareba, Mr. Octavian Aron Ngoda, Mr. James Tanguye, Ms. Emmanuela Mkalawa, and Ms. Adelina G. Gadiye from Muhimbili National Hospital (MNH).

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Lastly, I would also like to thank the International Medical Devices Regulators Forum (IMDRF), the World Health Organization (WHO), as well as the Medical Device Coordination Group (MDCG) established by the European Union (EU), for making their respective guidelines available for referencing."

The proper use and implementation of this protocol will ensure that the HIV antibody and/or antigen detection which have been designated to be of public health importance will perform clinically as claimed by manufacturers.

Dr. Kissa W. Mwamwitwa
Director for Medical Devices and Diagnostics Control

FOREWORD

The management of HIV remains a public health priority globally and within Tanzania, where accurate, reliable diagnostic solutions form the cornerstone of disease surveillance, treatment, and control. As the Tanzania Medicines and Medical Devices Authority (TMDA) continues to strengthen its regulatory mechanisms, ensuring the performance and safety of HIV serology assays used within the country is imperative.

This protocol for performance evaluation of HIV serology assays has been designed as a critical instrument in fulfilling that responsibility. It provides a systematic and robust approach. It outlines key parameters and procedures for evaluating the sensitivity, specificity, and operational effectiveness of HIV diagnostic tests. By adhering to internationally recognized standards, including ISO 20916, ISO 15189, and WHO guidelines. This will ensure that tests meet the rigorous criteria required for market authorization within Tanzania. It also addresses emerging complexities related to lot-to-lot variability, inter-reader variability, and operational characteristics, thereby ensuring comprehensive assessment.

Applicants, manufacturers, and evaluating laboratories are encouraged to carefully apply this protocol to generate high-quality data that supports regulatory decisions and the effective deployment of diagnostics in diverse healthcare settings. Furthermore, this document promotes harmonization of testing procedures, ensuring that the performance data obtained are consistent, reproducible, and credible.

In supporting our stakeholders, TMDA remains committed to continuously improving the performance evaluation process. We welcome constructive feedback and suggestions for the advancement of this protocol, as we collectively strive for enhanced diagnostic capabilities that respond to the evolving healthcare landscape.

This protocol is a testament to TMDA's commitment to safeguarding public health by ensuring that only effective and safe diagnostic devices reach the market, ultimately supporting efforts to mitigate the impact of HIV on our population.

Dr. Adam M. Fimbo
Director General

ABBREVIATIONS

EIA	-	Enzyme Immunoassay
HIV	-	Human Immunodeficiency Virus
IFU	-	Instructions for use
NAT	-	Nucleic Acid Test
PI	-	Principal Investigator
QC	-	Quality Control
TMDA	-	Tanzania Medicines and Medical Devices Authority

DEFINITION OF TERMS

Authority
Means the Tanzania Medicines and Medical Devices Authority or the acronym “TMDA” established under section 4(1) of the Act.

Applicant
Means any person or institution or company that applies formally for performance evaluation of IVD in Tanzania.

Clinical Performance
Means ability of an IVD to yield results that are correlated with a particular clinical condition/ physiological state in accordance to target population and intended use.

Conformity Assessment
Means the systematic examination of evidence generated and procedures undertaken by the manufacturer, under requirements established by the Authority, to determine that an IVD is safe and performs as intended by the manufacturer and, therefore, conforms to the Essential Principles of Safety and Performance of Medical Devices.

Diagnostic Sensitivity
Means the ability of a device to identify the presence of a target marker associated with a particular disease or condition.

Diagnostic Specificity
Means the ability of a device to recognize the absence of a target marker associated with a particular disease or condition.

Ethics Committee (EC)
Means an independent body composed of members with expertise in both scientific and non-scientific arenas which functions to ensure the protection of human rights and the well-being of research subjects based on six basic principles of autonomy, justice, beneficence, no maleficence, confidentiality and honesty.

Error rate
Means a measure of the degree of prediction error of a model made with respect to the true model.

In Vitro Diagnostics Devices
Means a device whether used alone or in combination, intended by the manufacturer for the in vitro examination of specimens derived from the human body and animals principally to provide information for diagnostic, monitoring or compatibility purposes. IVD include reagents, calibrators, control materials, specimen receptacles, software, and related instruments or apparatus or other articles and are used for example for the

following test purposes: diagnosis, aid to diagnosis, screening, monitoring, predisposition, prognosis, prediction and determination of physiological status.

Manufacturer
Means any person or a firm that is engaged in the manufacture of IVDs.

Negative likelihood ratio (LR-)
Means a probability that a person with the disease tested negative divided by the probability that a person without the disease tested negative.

Negative predictive value
Means the ratio of subjects truly diagnosed as negative to all those who had negative test results (including patients who were incorrectly diagnosed as healthy).

Performance evaluation
Means the assessment and analysis of data to establish or verify the performance (analytical performance and where applicable, clinical performance) of an IVD.

Positive predictive value
Means the ratio of patients truly diagnosed as positive to all those who had positive test results (including healthy subjects who were incorrectly diagnosed as patient).

Positive likelihood ratio (LR+)
Means probability that a positive test would be expected in a patient divided by the probability that a positive test would be expected in a patient without a disease.

1 Introduction

Accurate and reliable diagnosis of HIV is essential to effective treatment, care, and public health interventions. In-vitro diagnostic (IVD) tests for detecting HIV antibodies and antigens play a pivotal role in identifying infections, reducing transmission rates, and guiding treatment decisions. To ensure that these diagnostic tests meet the required performance standards, the Tanzania Medicines and Medical Devices Authority (TMDA) has developed this protocol, which outlines the procedures for conducting comprehensive laboratory evaluations of HIV serology assays.

This protocol provides a structured framework for evaluating the sensitivity, specificity, and operational performance of HIV diagnostic tests, including Enzyme Immunoassays (EIAs) and rapid diagnostic tests. By using a standardised HIV specimen reference panel and rigorous testing procedures, this protocol aims to establish the reliability of diagnostic tests under various conditions. These evaluations ensure that only products meeting predefined acceptance criteria are authorised for use in Tanzania. Moreover, it addresses key performance variables such as lot-to-lot consistency, inter-reader variability, and limits of acceptability.

The protocol aligns with international standards and best practices, offering manufacturers and applicants clear guidance to generate data that supports regulatory decision-making and the safe deployment of HIV diagnostics in clinical settings. Through effective implementation of this protocol, TMDA seeks to enhance public health outcomes by ensuring the availability of high-performing, reliable diagnostic tests.

2 Study objectives.

2.1 Overall objective

The overall objective is to evaluate and compare the accuracy of currently available HIV assays (including EIAs, rapid diagnostic tests and other formats) for detection of HIV antibody and/or HIV antigen against established performance criteria.

2.2 Specific objectives

The specific objectives of the performance evaluation are:

- To determine the sensitivity and specificity of currently available HIV assays (EIAs, rapid diagnostic tests and other formats) for the detection of antibodies to HIV as compared to a reference algorithm (two EIAs followed by a line immunoassay) and compare them to predefined acceptance criteria for market authorization by TMDA.
- To assess lot-to-lot variation
- To assess inter-reader variability (for subjectively read assays)
- To describe and assess the operational characteristics and ease of use of HIV serology assays.

3 Study implementation.

3.1 Performance evaluation laboratory

The laboratory shall hold one of the following certifications for quality management within the laboratory: ISO 17025 *General requirements for the competence of testing and calibration laboratories*, ISO 15189 *Medical laboratories: Requirements for quality and competence* or equivalent.

The Head of the Laboratory will act as the Principal Investigator (PI) for the work performed by the laboratory.

3.2 Training, performance evaluation and supervision

The following issues are key to minimizing error and maximizing the value of this performance evaluation:

- The PI will be responsible for training the laboratory professionals on the details of the evaluation protocol and on the performance of each assay undergoing evaluation.
- Only those personnel who have received specific training for a particular assay evaluation will be employed.
- Accurate record keeping is crucial to the success of the evaluation and the PI will be responsible for ensuring that all data required for the evaluation are recorded on the agreed data collection sheets and are accurate and up to date.
- It is important to plan work in advance and follow standard operating procedures as prepared and controlled by the laboratory.
- To reduce the risk of adding an incorrect specimen to a test device/well, before starting the test run, the operator will prepare worksheets and label all tubes, dilution vessels, test devices or plates with the specimen's unique number.
- Because objective, machine-generated, permanent results for simple/rapid diagnostic tests are not feasible, it is essential that the PI emphasizes to the operator performing the tests the need for accurate recording of results and recordkeeping.

- To minimize the risk of error, it is recommended that the results are read and recorded independently by three trained staff members.
- To allow immediate correction of erroneous recording of results (rather than differences in visual interpretation), the PI or designee should assess the results as soon as possible to allow her/him to return to the original test device to investigate apparently discordant readings.
- For the performance evaluations performed at the laboratory, at least one representative result from both Hepatitis B antigen positive and negative specimens will also be recorded by taking electronic images. Unexpected test results will also be digitally recorded as well an image of the instructions for use.

3.3 Safety

HIV, hepatitis B and hepatitis C and other viruses are transmissible by blood and body fluids. Therefore, all types of specimens (including venous and capillary whole blood, serum/plasma, oral fluid, etc.) must be handled as potentially infectious. Appropriate precautions to minimize infectious hazards must be taken at all stages from the collection of specimens to the disposal of used materials from the laboratory. The WHO Guidelines on HIV Safety Precautions, and Guidelines for the Safe Transport of Specimens (WHO/EMC/97.3) and the evaluating site's guidelines on laboratory safety should be followed carefully by the laboratory staff.

3.4 Storage of assays

All reagents must be stored as indicated in the instructions for use. Some assays may not need refrigeration. If refrigerated storage space is inadequate to store the entire test kit, they may be divided so that labile reagents can be refrigerated separately from the non-labile supplies. Calibrated thermometers are placed at each location where reagents and specimens are stored, i.e. ambient, refrigerator and freezer. Temperatures are recorded daily on the laboratory temperature logs. The lot numbers of the test kits received/used, and their expiry dates are recorded on the individual run worksheets.

Two separate production lots (with different lot numbers and different expiry dates) will be requested for evaluation, according to the following definition of a lot[1]: *"The amount of material that is uniform in its properties and has been produced in one process or series of processes. The material can be either starting material, intermediate material or finished product.*" Each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents. Furthermore, lots must be sourced from a representative production run and not produced especially for the purpose of this evaluation. TMDA will verify this information before the product assessment has been finalized.

4 Specimens

4.1 Clinical performance specimen panel

A panel of 1200 well-characterized serum/plasma clinical specimen, including 470 anti-HIV positive specimens and 730 anti-HIV negative specimens, will be used for this evaluation. Efforts will be made to include also anti-HIV2 positive specimens.

4.2 Specimen collection and storage

As much as possible, specimens used in this evaluation will be representative of the intended use population, i.e. from clients screened for HIV infection in different regions in Tanzania.

The panel may consist of the following specimens:

- Left-over specimens from clients attending HIV testing services.
- Left-over specimens from known HIV-positive patients from an HIV clinic. In this case, efforts will be made to ensure that patients are not yet on treatment or have not been on treatment for more than one year to ensure that their antibody profile is not affected.
- Rejected blood units from blood donation centers, rejected because they were found to be infected with blood borne pathogens including HIV, Hepatitis B, Hepatitis C, Syphilis etc.

After removing all the specimen identifiers, specimens are assigned a unique identification number at the laboratory. Once the specimens have been processed and labelled, they are aliquoted and frozen immediately at -70°C. During the period of testing the specimens are stored at 2 - 8 °C and this time period does not exceed one week. After the completion of testing, they are again stored at -70 °C.

The number of freeze-thaw cycles should be recorded. Each aliquot should not undergo more than five freeze/thaw cycles, as this has been shown not to affect the stability of antibodies. For assays also detecting antigens (4th generation assays), aliquots with a maximum of 3 freeze/thaw cycles will be used. If a lower number of freeze/thaw cycles is requested by the manufacturer, data should be provided to TMDA to support the request.

4.3 Characterization of the HIV specimen reference panel

The HIV clinical specimen panel shall be characterized using a standardized combination of assays i.e. a testing algorithm (Figure 1). These results are used to determine the HIV status of each specimen for the purpose of the performance evaluation. Use of any other combination of assays for characterization of the HIV specimen evaluation panel shall be communicated, discussed and agreed with TMDA beforehand.

Initially, each specimen is tested on Genscreen™ ULTRA HIV Ag-Ab (Bio-Rad) and AiD™ anti-HIV 1+2 ELISA (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd) in parallel. *Other combinations of EIAs may be acceptable if they meet with the following principles: both EIAs should be WHO prequalified and there should be one 3rd generation and one 4th generation assays.*

Specimens that are non-reactive on both EIAs are not further tested and are assigned anti-HIV negative.

Specimens with discrepant EIA results AND those with dually reactive results on both EIAs are tested with the INNO-LIA™ HIV I/II Score (Fujirebio) line immunoassay.

Specimens that are negative by line immunoassay are further tested on Innotest® HIV Antigen mAb (Fujirebio) EIA and, if found non-reactive, then are assigned anti-HIV negative. If found to be neutralizable for HIV-1 antigen, the specimen is considered HIV-1 antigen

positive and anti-HIV negative and is retained for the evaluation of 4th generation assay but not for 3rd generation assays.

Specimens that are indeterminate by line immunoassay are further tested on Innotest® HIV Antigen mAb (Fujirebio) EIA and if found non-reactive then are excluded from the panel. Specimens that are reactive for antigen (and neutralisable) are assigned as HIV-1 antigen positive and anti-HIV inconclusive. These specimens are retained for the evaluation of 4th generation assay but not for 3rd generation assays.

Specimens that are positive by line immunoassay are assigned as anti-HIV-1 positive or anti-HIV-2 positive. Those specimens that cannot be discriminated (i.e. anti-HIV positive) are further tested on the NEW LAV II Blot (BioRad Laboratories). Specimens that are indeterminate or negative by the NEW LAV II Blot are assigned as anti-HIV-1 positive. Specimens that are positive by the NEW LAV II Blot are assigned as anti-HIV positive.

5 Laboratory testing

Regarding evaluating rapid diagnostic tests, a 'test run' is defined as a consecutive run of simple/rapid diagnostic tests of the same production lot performed during the same 'session'. A 'testing session' might be considered to be a morning or afternoon.

Kit controls, if available, and external quality controls are tested with (at the beginning of) each test run review of the instructions for use

Each product under evaluation is used strictly in accordance with the instructions for use (IFU) issued by the manufacturer. The laboratory will send a hard or electronic copy of the IFU to TMDA upon delivery of the test kits and prior to the commencement of the laboratory evaluation. The IFU must be reviewed against the IFU submitted to TMDA as part of the dossier assessment for the prequalification assessment. If the IFU has been updated since dossier submission, a letter from the manufacturer detailing changes made must be sent to TMDA prior to the laboratory evaluation commencing.

5.1 Review of the instructions for use

Each product under evaluation is used strictly in accordance with the instructions for use (IFU) issued by the manufacturer. The laboratory will send a hard or electronic copy of the IFU to TMDA upon delivery of the test kits and prior to the commencement of the laboratory evaluation. The IFU must be reviewed against the IFU submitted to TMDA as part of the dossier assessment for market authorization procedure. If the IFU has been updated since dossier submission, a letter from the manufacturer detailing changes made must be sent to TMDA prior to the laboratory evaluation commencing.

5.2 Clinical performance panel

The specimen reference panel is run in order that approximately one half of the specimen panel will be run with the one lot and the other half of the panel with the other lot. The specimens of the HIV specimen reference panel should initially be tested in singular and in a blinded manner.

Specimens with invalid result should be retested in singular on the same lot.

Specimens which are found to be indeterminate by the criteria stated in the instructions for use (e.g. grey zone for EIAs) should be retested in duplicate on the same lot number of assay and singular on the other lot. In the case that the testing result cannot be resolved after all testing, the specimen is to be called indeterminate.

Specimens with results discrepant from the reference result are retested in duplicate using the same lot number by the same operator. The results that occur two out of three times are recorded as the test result. If the result is again discrepant, the specimen is retested on a second lot number, if available. If the result on the second lot is concordant with the reference result, no further testing is required. If the result is still discrepant from the reference results, the result is recorded as is.

In all cases of repeat testing, all results (initial and repeat testing) should be recorded.

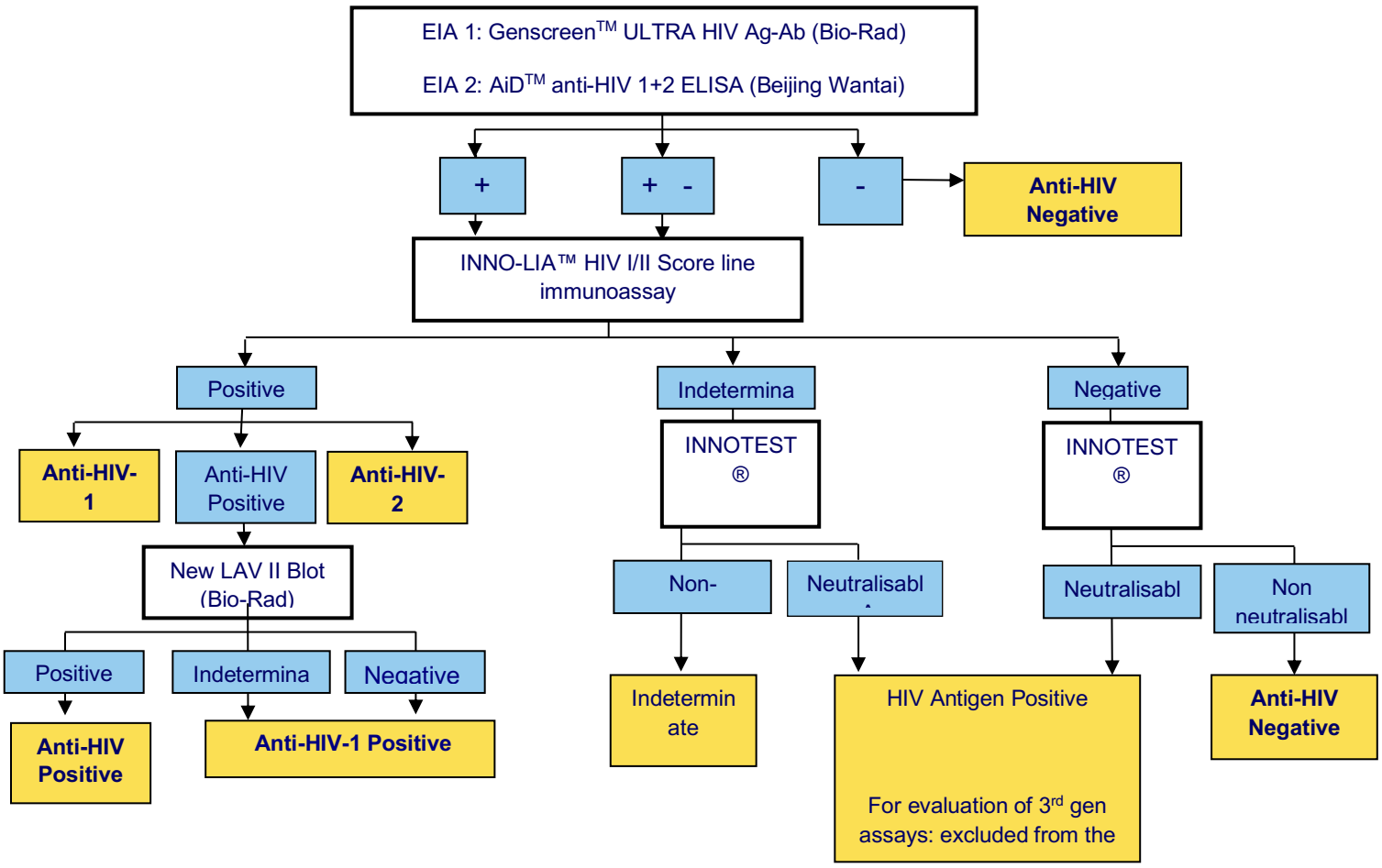

Figure 1. Testing algorithm for characterization of the TMDA HIV specimen reference panel (serum/plasma specimens)

5.3 Criteria to continue testing.

After testing the first 50 specimens (for rapid tests) or the first plate (for EIAs, approximately 90 samples), an interim analysis is done, and results are communicated to TMDA. If less than 90% of the results are concordant with the reference results, then the evaluation is stopped, and troubleshooting should be done by TMDA and the manufacturer.

5.4 Lot-to-lot variation panel

Lot-to-lot variation is assessed by testing the same ten dilution series, as described in section 4, on two separate production lots of the assay under evaluation in the same testing session.

5.5 Interpretation of results

The interpretation of results for each assay under evaluation is made strictly according to the manufacturers' instructions within the IFU. Invalid test results are recorded on the data collection sheets including where the control line does not appear or in any other way the test result is invalid as defined by the IFU. For test results that are indeterminate according to the IFU, the results are recorded on data collection sheets.

Visual interpretation of results of subjectively read assays is made independently by three readers (without the knowledge of the other two sets of results and blinded to the reference result for the specimen) and entered onto the data collection sheets. These results are compared by the operator carrying out the assay so that any mistakes may be identified and rectified immediately. Should recording errors be identified, both the original and corrected result are recorded and initialed by the reader. When the three readers interpret the results differently from each other (i.e. reactive/non-reactive), the consensus is recorded as that interpretation which occurs two out of three times. In cases where all three interpretations are different, the result is recorded as indeterminate.

5.6 Recording test results

All test results are recorded on standardized test result worksheets and then entered in a Microsoft Excel spreadsheet for further data analysis.

Where applicable for EIAs, test results will be recorded electronically directly from the plate reader and then entered in a Microsoft Excel spreadsheet for further data analysis. Printed records will also be generated from the EIA reader, and these will be used to cross-check a sample of the imported data.

For subjectively read assays such as rapid diagnostic tests the intensity of band/line/spot is additionally entered into the data collection sheet. The intensity rating system reads as described in Table 1.

Table 1. Result legend for subjectively read assays.

Scoring index	Intensity reading scale

0	Non-reactive
1	Very Weak
2	Weak
3	Medium to Strong Reactivity
7	Invalid

6 Quality control and interpretation of test results

6.1 Test kit controls.

Manufacturer/Applicant supplied positive and negative test kit controls will be run as indicated in the IFU for all test formats included in each test run for EIAs and at the commencement of each testing session for rapid diagnostic tests and other formats. Where positive and negative test kit controls are not supplied by the manufacturer/applicant, as will be the case for many rapid diagnostic tests, the external quality control specimen will act at the control specimen.

6.2 Internal control lines for rapid diagnostic tests

Generally, rapid diagnostic tests contain a control band, line or spot to determine migration of the reagents, or the sample has occurred. Most control bands/lines/spots will become visible with the addition of reagent (i.e. buffer). However, some rapid diagnostic tests will contain a control band/line that becomes visible with addition of specimen (i.e. presence of IgG). It is imperative that the exact nature of the control band/line is ascertained and included in the report. An experiment is performed to verify this point, if not explicitly mentioned in the IFU.

6.3 External quality control specimen

The laboratory will supply an external quality control (QC) specimen which is tested in singular at the beginning of each test session for rapid diagnostic tests. The QC specimen represents a weakly reactive Hepatitis B positive sample. The QC specimen is prepared by the laboratory or acquired commercially, depending on the assay under evaluation.

6.4 Competency panels

User competency must be established for each assay by each operator before the evaluation commences. This may be established at the time of assay demonstration by the manufacturer or for training purposes.

6.5 Limits of acceptability

All results on test kits controls and QC specimens are entered on the data collection sheets. Should the test kit controls or the QC specimen not give results within the expected ranges, evaluation testing on that assay is suspended until the cause has been identified and a satisfactory solution identified. Such problems must be communicated immediately to

TMDA and recorded on the data sheets. The PI is responsible for carefully checking all data entry forms for legibility, accuracy and completeness.1010

7 Analysis of data

7.1 Invalid devices

The number of invalid test runs (if EIA) is recorded as the number of invalid runs as a percentage of the total number of runs performed for clinical specimens only (excluding the lot variation panels).

The number of invalid devices (if rapid diagnostic test or other format) is recorded as the number of invalid test devices as a percentage of the total number of devices used for the evaluation testing with clinical specimens (excluding, lot to lot variation panels).

Invalid results may mean invalid test results as defined by the instructions for use such as where the control line/band/spot does not appear or invalid due to obviously defective test device or defective transfer pipette.

7.2 Inter-reader variability

The inter-reader variability is calculated when test results must be read without any objective reading instruments i.e. rapid diagnostic tests. Three persons independently interpret each test result. The inter-reader variability is expressed as the percentage of specimens for which initial test results are differently interpreted (i.e. reactive or non-reactive or indeterminate, if applicable) by the independent readers for the clinical specimens (excluding commercially acquired panels, TMDA reference preparations, culture supernatant panels, lot to lot variation panels).

Inter-reader variability is assessed for each test band i.e. HIV-1 and HIV-2 test bands in discriminatory HIV-1/2 antibody detection assays, and HIV-1/2 antibody and HIV-1 p24 antigen test bands for antibody/antigen detection assays.

7.3 Clinical performance characteristics

The following strategies are used to calculate the clinical performance characteristics by comparing the results of the assay under evaluation and reference testing results on the clinical specimen panel.

Table 2. 2 x 2 table for calculation of performance characteristics

	Reference testing results			
Results of assay under evaluation		HIV-positive	HIV-negative	Total
	Reactive	a (true positives)	b (false positives)	a + b
	Non-reactive	c (false negatives)	d (true negatives)	c + d
	Total	a + c	b + d	a+b+c+d

7.4 Sensitivity

Sensitivity is the ability of the assay under evaluation to detect correctly specimens that contain the analyte (reference results positive). Thus, sensitivity is the number of true positive specimens identified by the assay under evaluation as positive (a), divided by the number of specimens identified by the reference assays as positive (a+c), expressed as a percentage.

$$\textbf{Sensitivity} = \frac{a}{a+c} \; x100\%$$

7.5 Specificity

Specificity is the ability of the assay under evaluation to detect correctly specimens that do not contain the analyte (reference results negative). Thus, specificity is the number of true negative specimens identified by the assay under evaluation as negative (d), divided by the number of specimens identified by the reference assays as negative (b+d), expressed as a percentage.

$$\textbf{Specificity} = \frac{d}{b+d} \; x100\%$$

7.6 Confidence intervals

The exact 95% confidence intervals for binomial proportions are calculated for both sensitivity and specificity.

7.7 Initial and final sensitivity and specificity

The initial sensitivity and specificity are calculated based on the initial results obtained for the assay under evaluation (except for invalid results, for which the result of repeated testing is used). If the initial result is indeterminate, then the specimen is excluded from this analysis. The proportion of initial indeterminate results is reported.

The final sensitivity and specificity values are calculated taking into consideration the repeat testing performed on the same lot and further testing second lot of the assay under evaluation, if applicable (i.e. for specimens with initial indeterminate or discrepant results).

7.8 HIV-1 and HIV-2 discriminatory assays

For assays with the ability to discriminate between HIV-1 and HIV-2, results will also be reported using Table 3. However, sensitivity and specificity will be assessed globally for detection of HIV, as described above.

The number of HIV-1 positive specimens identified as HIV-1 and HIV-2 specimens identified as HIV-2 will be reported. In addition, the number of false-reactive HIV-2 results among HIV-1 positive specimens and false-reactive HIV-1 results among HIV-2 positive specimens will be reported.

Table 3. 2 x 2 table for reporting results for HIV-1 and HIV-2 discriminatory assays

	Reference testing results			
Results of assay under evaluation		HIV-1 positive	HIV-2 positive	HIV-negative
	Reactive (HIV-1)			

	Reactive (HIV-2)			
	Reactive (HIV-1+ HIV-2)			
	Non-reactive			
	Total			

8 Analytical performance characteristics

8.1 Results from lot-to-lot variation panel

The results of the lot-to-lot panel for the two production lots are compared and a variation of +/- 1 dilution series is considered acceptable. The number of series with acceptable and non-acceptable variation is reported.

8.2 Laboratory professional's appraisal

The technical aspects of the assay under evaluation are assessed by the Laboratory professional(s) who performed the testing. These assessments, along with other selected assay characteristics, contribute to an overall appraisal of each assay's suitability for use in small laboratories. To enable comparison between assays, a scoring system is used to rate specified operational characteristics (Annex 1).

8.3 Report preparation and dissemination.

The preliminary data analysis and drafting of the report will be carried out by the evaluating laboratory according to pre-defined report templates.

The draft report will be shared simultaneously with TMDA and the manufacturer.

8.4 Acceptance criteria

The following criteria will be used to assess the assay under evaluation. Other parameters included in this evaluation are provided for information but are not used as pass/fail criteria for this assessment.

Table 4. Minimum acceptable performance for HIV serology assays in the TMDA prequalification performance evaluation

Parameter	**EIA**	**Rapid diagnostic tests**
Initial sensitivity estimate	100%	≥ 99%
Final specificity estimate	≥ 98%	≥ 98%
Inter-reader variability	N/A	≤5%
Invalid rate	<5%	<5%

9 Materials and supplies

The manufacturers of products will provide the products and any equipment necessary for the evaluation free of charge.

Table 5. Number of tests required to perform this evaluation.

	Lot A	**Lot B**
Clinical panel	600	600

Lot-to-lot variation	100	100
Total	700	700
Total + 20% for controls and repeats (for antibody detecting assays)		

10 Roles and responsibilities

10.1 Responsibilities of the Evaluating Laboratory

i. Ensure availability of HIV specimen reference panel, lot-to-lot variation, panel;
ii. Conducting the performance evaluation in accordance with internationally recognized best practice;
iii. Preparation of QC specimens and proficiency panels;
iv. Preparation of draft report on laboratory evaluation;
v. Advising TMDA on operational characteristics of assays evaluated.

All source data, data analysis records and all correspondence are retained and archived for a period of at least ten years.

10.2 Responsibilities of TMDA

i. Technical advice to the PI;
ii. Technical and administrative management of the laboratory evaluation;
iii. Verification of the draft report, seeking of comments from manufacturer in case of List one laboratories;
iv. Preparation and dissemination of the final report;
v. Formal contacts with authorized contacts of the manufacturers.

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WHO. Guidance for procurement of in vitro diagnostics and related laboratory items and equipment.https://iris.who.int/bitstream/handle/10665/255577/9789241512558-eng.pdf?sequence=1

EN 13612:2002 Performance evaluation of in vitro diagnostic medical devices
ISO 17025 (General requirements for the competence of testing and calibration laboratories)
ISO15189 (Medical laboratories — Particular requirements for quality and competence)

11 Annex 1: Operational characteristics and ease of use

This assay requires/does not require laboratory equipment and can/cannot be performed in laboratories with limited facilities or in non-laboratory settings. *If applicable, add specifics on why it cannot be used in laboratories with limited facilities: e.g.* the instrument requires a stable source of electricity and significant physical space. Furthermore, training and implementation of good laboratory practice is essential to obtaining accurate results. *If technical support was needed during evaluation:* Adequate technical support from manufacturer or representative is critical.

The assay was found easy to use / not easy to use by the operators performing the evaluation. *If applicable, add specific information provided in comments in the ease of use table.*

For RDTs (and ELISA)

Key operational characteristics	
Number of steps*	X steps in total X steps with precision pipetting *(only for serum/plasma)*
Time to result	X minutes
Endpoint stability (interval)	X minutes (the test can be read between xx and xx minutes after addition of specimen/diluent)
Internal QC	Yes/no, *insert brief description. [The test has an internal control line. The presence of the control line indicates that migration of liquid has occurred; however, it does not guarantee that the correct specimen type or volume was added or that the test procedure was followed correctly.]*

** Definition: each action required to obtain a result (excluding specimen collection, device preparation – opening the pouch), e.g. for RDTs: add specimen, add buffer (2 steps).*

For instrument-based assays

Key operational characteristics	
Number of steps for one specimen*	X steps in total X steps with precision pipetting
Number of steps for instrument management**	X steps per run/day
Time to result for one test/run	X minutes
Operator hands-on time for one test/run	X minutes
Level of automation	
Quality controls	QC are/are not provided by the manufacturer and should be purchased separately. *Add information on type of QC (eg. high positive, low positive, negative)*
Operating temperature	xx- xx °C, any comments on temperature stability of conducting the test.
Result display and connectivity	Results are displayed on the instrument / connected computer. They may be printed using

	a standard/specific printer. The results can be exported to the laboratory information system and other health information systems.
Power sources	Main power / Battery / Solar power The use of a UPS is recommended, as stable electricity is required
Biosafety *(outside of infectious specimen handling)*	Operators reported no biosafety concerns for the user. *Add information if applicable*
Waste	The volume of liquid was is approx. xx per test/run. The volume of solid waste is approx. xx per test/ run. Waste disposal requires / does not require specific measures in addition to usual laboratory biohazard waste disposal procedures. *Add information if applicable.*
Calibration	Calibrators are/are not provided by the manufacturer and should be purchased separately. *Add frequency of calibration recommended.*
Maintenance	Daily / Weekly / Monthly / Yearly / No maintenance is required.
Other specific requirements	*If applicable (eg. space requirements, weight to surface area ratio, installation by manufacturer)*