TSS-3

Malaria rapid diagnostic tests, second edition

Technical specifications series for submission to WHO prequalification – diagnostic assessment

World Health Organization

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Declarations of interest

All external experts and meeting participants submitted to WHO a declaration of interest disclosing potential conflicts of interest that might affect, or might reasonably be perceived to affect, their objectivity and independence in relation to the subject matter of the guidance. WHO reviewed each of those and had concluded that none could give rise to a potential or reasonably perceived conflict of interest related to the subjects discussed covered by the guidance.

All the declarations were made known to all participants at the beginning of the meeting.

All the experts participated in their individual capacities and not as representatives of their countries, governments or organizations.

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[1] Via teleconference
[2] Via teleconference
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Abbreviations

ANOVA	analysis of variance
ELISA	Enzyme linked immunoassay
HAMA	human anti-mouse antibody
HRP2/3	histidine-rich protein 2/3
IFU	instructions for use
IgG, IgM	immunoglobulins G and M
IMDRF	International Medical Device Regulators Forum
IMDRF ToC	International Medical Device Regulators Forum “Table of Contents”
IVD	in vitro diagnostic medical device
LDH	lactate dehydrogenase
LoD	limit of detection
NIBSC	The National Institute for Biological Standards and Control
PCR	polymerase chain reaction
Pf	*Plasmodium falciparum*
pLDH	pan lactate dehydrogenase
POC	point of care
Pv	*Plasmodium vivax*
RDTs	rapid diagnostic tests
RPS WG	regulatory product submission working group (an IMDRF working group)
Spp.	species
TGS	WHO prequalification Technical guidance series
TSS	WHO prequalification Technical specifications series
US FDA	United States Food and Drug Administration
WHO	World Health Organization

A. Introduction

The purpose of this document is to provide technical guidance to in vitro diagnostic medical device (IVD) manufacturers that intend to seek WHO prequalification of IVDs for the detection, in blood, of antigens produced by Plasmodium (malaria) species. For the purposes of WHO prequalification, this document applies only to rapid diagnostic tests (RDTs) intended to diagnose malaria infection in symptomatic patients.

For the purpose of this document, the verbal forms used follow the usage described below:

- "shall" indicates that the manufacturer is required to comply with the technical specifications.
- "should" indicates that the manufacturer is recommended to comply with the technical specifications, but it is not a requirement.
- "may" indicates that the technical specifications are a suggested method to undertake the testing, but it is not a requirement.

A documented justification and rationale shall be provided by the manufacturer when the WHO prequalification submission does not comply with the required technical specifications outlined in this document.

Minimum performance requirements for WHO prequalification are summarized in this document and, where possible, are aligned with published guidance, standards and/or regulatory documents. Although references to source documents are provided, in some cases WHO prequalification has additional requirements. External quality controls are outside the scope of this document.

The analytical sensitivity of the RDT shall be sufficient to allow detection of a minimal clinically significant antigenemia, established as at least a 75% "panel detection score" for low parasite density samples (200 parasites/µL) from the product testing evaluation panel for the detection of *Plasmodium falciparum* Histidine Rich Protein 2 (Pf-HRP2) expressing and nonHRP2 expressing panels and, if applicable, for *P. vivax* (see the WHO Global malaria programme selection and procurement criteria for malaria RDTs (***1***) and the summary results of WHO product testing of malaria RDTs: round 1-8 (***2***)).

For WHO prequalification purposes, manufacturers shall provide evidence in support of the clinical performance of an IVD, demonstrating that reasonable steps have been taken to ensure that a routinely manufactured IVD, when correctly operated by the intended user and assessed with a representative spectrum of patients who will receive the test in practice, will detect the target analyte and fulfil its intended use (***3***).

WHO prequalification requirements summarized in this document do not extend to the demonstration of clinical utility, i.e., the effectiveness and/or benefits of an IVD, relative to and/or in combination with other measures, as a tool to inform clinical intervention in a given population or healthcare setting. To demonstrate clinical utility, a separate set of studies is required. Clinical utility studies usually inform programmatic strategy and are thus the responsibility of programme managers, ministries of health and other related bodies in

individual WHO Member States. Such studies do not fall under the scope of WHO prequalification.

B. Other guidance documents

This document should be read in conjunction with other WHO guidance documentation, including:

WHO prequalification documents:

- Technical guidance series (TGS) for WHO prequalification – diagnostic assessment available (*4*)
- Instructions for compilation of a product dossier, WHO document PQDx_018. (*5*).

WHO Global Malaria Programme documents:

- WHO Global Malaria Programme (GMP) Selecting and procuring malaria RDTs (*1*)
- Quality and safety practices for malaria rapid testing services (*6*)
- Methods manual for laboratory quality control testing of malaria RDTs (*7*)
- False-negative RDT results and implications of new reports of *P. falciparum* histidine-rich protein 2/3 gene deletions. (*8*)

C. Performance principles for WHO prequalification

C.1 Intended use

An IVD intended for WHO prequalification shall be accompanied by a sufficiently detailed intended use statement. This should allow an understanding of at least the following:

- The type of assay (e.g. lateral-flow or flow-through immunochromatographic test);
- What the IVD measures or detects: (e.g. to detect *P. falciparum* HRP2 antigen, *P. falciparum* LDH antigen, *P. vivax* LDH antigen; pan LDH));
- The function of the IVD; (e.g., assist in the diagnosis of malaria by detecting evidence of malaria parasites (antigens) in human blood, differentiation between *Plasmodium falciparum* and non-*Plasmodium falciparum* species);
- The specific disorder, condition or risk factor of interest that is intended to detect, define or differentiate (e.g. to diagnose malaria infection);
- Whether or not it includes automated components or is intended to be used with automated instruments;
- The testing population for which the functions are intended (e.g., paediatric testing, symptomatic patients);
- How the results are reported (e.g., qualitative test);
- The intended use environment (e.g. at point of care (POC), in a community setting, or in a laboratory setting);
- The intended user (laboratory professionals[4], healthcare workers or trained lay providers[5]);

[4] Medical technologists, medical laboratory technicians or similar, who have received a formal professional or paraprofessional certificate or tertiary education degree.
[5] Any person who performs functions related to healthcare delivery and has been trained to deliver specific services but has received no formal professional or paraprofessional certification or tertiary education degree.

- The intended specimen type (e.g. capillary or venous blood), including specimen source, matrix, time and collection methods (e.g. safety lancets for capillary whole blood collection, transfer device); and
- Any limitations to the intended use.

C.2 Diversity of specimen types, users and testing environments and impact on required studies

For WHO prequalification submission, clinical performance studies should be conducted using the specimen types most likely to be used in resource-limited WHO Member States (i.e. capillary whole blood) and are claimed in the instructions for use. If this is not possible, data should be presented to show the equivalence between specimen types used in performance studies.

Prequalified IVDs in low- and middle-income countries are likely to be used by laboratory professionals and at point-of-care by healthcare workers or trained lay providers. Depending on the intended use of a RDT, performance studies shall be designed to take into account not only the diversity of knowledge and skills across the population of RDT users, but also the likely operational settings in which testing will occur. For example, studies that comprise the testing of left-over/repository specimens by research and development staff at a manufacturer's facility would, on their own, be considered insufficient to meet many of the performance requirements outlined in this document.

Malaria testing often occurs in conditions of high temperature (>35 °C) and humidity. It is a manufacturer's responsibility to ensure that the risk assessment for an IVD reflects the intended operational settings and testing population.

C.3 Applicability of supporting evidence to IVD under review

The true *Plasmodium* status of a specimen shall be determined using microscopy, and differentiation of *Plasmodium* species by using a suitable molecular method, for which a scientific justification shall be provided.

Estimation (and reporting) of IVD performance shall include the rate of invalid test results (where 'invalid' is a result interpretation defined in the instructions for use).

Analytical performance studies should be undertaken with natural specimens. Contrived specimens (e.g. where negative human matrix has been spiked with *Plasmodium* reactive specimens) should only be used in the submitted studies if a scientific justification is provided. The use of recombinant *Plasmodium* antigens should be avoided. Clinical performance studies shall be based on testing in natural specimens only.

Performance studies shall be undertaken using the specific final, locked-down version IVD intended to be submitted for WHO prequalification. Where this is not possible (e.g. because of design variation), a justification for use of earlier versions of the IVD shall be provided, however additional supporting evidence may be required. This may occur following minor design variations or IFU changes where no impact on performance has been demonstrated (see WHO document Reportable changes to a WHO prequalified in vitro diagnostic medical device. (*8*)

For RDTs that include a claim for detection of multiple antigens and/or species, evidence of performance shall be provided for each claimed antigen and/or species. RDTs claiming to provide 'pan'-specific detection of 'malaria' are expected to detect all known pathogenic species of *Plasmodium*, i.e.: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. Full characterisation of all species is required for parasites utilized in the analytical studies, including quantification by ELISA (please refer to the Global malaria programme methods manual for additional information ((7) and reporting should clearly state results for each species. For RDTs with a pan-species (e.g. pan-LDH) detection claim, performance characteristics shall be established for all relevant Plasmodium species; if this is not done, the instructions for use must report this limitation as warning to the user.

It is important to note that, depending on the design of an IVD, evidence generated in a similar, related product will not be sufficient to support performance claims in an IVD submitted for WHO prequalification. For example, evidence of *Plasmodium falciparum* HRP2 (Pf-HRP2) detection in a Pf-HRP2-only RDT will not be accepted as evidence to support Pf-HRP2 detection in a subsequent dual-detection version of the RDT designed to detect both Pf-HRP2 and *Plasmodium falciparum* lactate dehydrogenase (Pf-LDH).

D. Table of Requirements

WHO requires that a product dossier be submitted in the "Table of Contents" (ToC) format, described in the International Medical Device Regulators Forum (IMDRF) document IMDRF/RPS WG/N13 Final:2024 (Edition 4). (*9*) In the tables below, the chapters and subheadings are labelled and numbered according to IMDRF ToC format. As the IMDRF ToC is comprehensive in nature, not all subheadings are required for WHO prequalification and are excluded. As a result, the subheading numbering in the tables below is not always continuous (e.g., 3.05.10, 3.06.04, etc). This has been done to maintain consistency between sections required in a product dossier for WHO prequalification assessment and the corresponding numbering defined in the IMDRF ToC format.

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Part 1 IMDRF ToC Chapter 3: Analytical performance and other evidence

IMDRF ToC chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
3.05 Analytical performance			
3.05.01 Stability of specimen(s)			
Specimen stability	Real time studies shall be conducted taking into account: 1. Storage conditions (duration at different temperatures and variation in humidity, temperature limits, freeze/thaw cycles). 2. Transport conditions, where applicable. 3. Intended use (see note 1). 4. Specimen collection and/or transfer devices, whether these contain anticoagulants and whether they can be sealed. 5. Testing should include a minimum of 10 specimens from different individuals. • Specimens shall be weakly reactive: 2—3 x limit of detection (LOD).	1. Particular attention shall be paid to the length of time likely to elapse between specimen collection and its addition to the IVD in the settings where this IVD may be used.	
3.05.02 Validation of specimens			
3.05.02a Demonstration of equivalence between specimen types	1. For each claimed specimen type, testing shall be conducted in at least: • 25 *Plasmodium* negative specimens. • 25 *Plasmodium* positive specimens. 2. Equivalence shall be determined for each claimed *Plasmodium* antigen and/or species, as appropriate.	1. The relationship between IVD performance in claimed specimen types and reference materials used for analytical performance studies shall be clearly established. The design of subsequent studies shall then take that relationship into account. 2. If there is no equivalence between claimed specimen types, then the impact that this will have on each	TGS-3 (*10*)

IMDRF ToC chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
3.05.02b Demonstration of equivalence of claimed anticoagulants	1. For each claimed anticoagulant, testing shall be conducted in at least: • 25 *Plasmodium* negative specimens. • 25 *Plasmodium* positive specimens (see note 3). 2. Equivalence shall be determined for each claimed *Plasmodium* antigen.	subsequent performance claim shall be fully understood and described. *Example: an IVD intended for testing whole blood for which the measuring range is estimated using panels of serum/plasma specimens.* • *The relationship between analytical sensitivity in serum/plasma to that of the same characteristic in whole blood shall be understood.* • *This may be achieved by comparing end-point dilution series of matched positive patient specimens (whole blood vs. serum/plasma collected from the same patient at the same time for testing) or may be determined as part of clinical performance studies.* 3. Positive specimens shall be chosen so that a majority are near the LOD.	
3.05.03 Metrological traceability of calibrators and control material values			
Metrological traceability of calibrators and control material values	1. The traceability of an external control to a validated reference material shall be demonstrated (e.g. to WHO First WHO International Standard for *Plasmodium falciparum* antigens NIBSC code: 16/376).	1. WHO encourages the use of external/quality control specimens which shall be traceable to a validated reference material and demonstrate whether a test result is valid.	
3.05.04 Accuracy of measurement			
3.05.04.02 Precision (repeatability & reproducibility)	1. Both repeatability (within-condition – see note 1) and reproducibility (between-condition – see note 1) shall be estimated by replicate testing of end-point dilutions of several analyte-positive specimens (see note 2, 4).	1. E.g. within- or between-run, -lot, -day, -operator, -site, etc. 2. Precision shall be determined for each analyte for which detection is claimed (e.g. Pf-HRP2, pLDH, etc.).	CLSI EP05-A3 (*11*) CLSI EP17 (*12*) EN 13612:2002 (*13*)

IMDRF ToC chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	2. Specimens chosen for the testing panel shall include panel members that reflect the main specimen types intended for use with the IVD (e.g. capillary or venous whole blood). 3. Each panel member shall be tested: • Using 3 different lots (see note 9). • Over 5 days (not necessarily consecutive) with one run per day (alternating morning/afternoon). • At each of 3 different testing sites. 4. The effect of operator-to-operator variation on IVD performance shall be included as part of the precision studies (see also note 10). Testing shall be performed: • By users representative of intended users (note 11). • Unassisted. • Using only those materials provided with the IVD (e.g. lancets, transfer pipets, instructions for use, labels and other instructional materials).	3. Where possible, the testing panel should be the same for all operators, lots and sites. 4. Low-reactivity specimens shall be chosen that are sufficiently close to the assay LOD to allow changes in IVD sensitivity to be detected. 5. The numbers of invalid tests shall be reported. 6. Lots shall be composed of different batches of critical components. 7. Results shall be statistically analyzed using analysis of variance (ANOVA) techniques to identify and isolate the sources and extent of any variance). 8. In addition to ANOVA, the percentage of correctly identified, incorrectly identified and invalid results shall be tabulated for each specimen and be separately stratified according to each of site, lot, etc. This type of analysis is especially important for rapid tests that may not have any numerical values for ANOVA analysis. 9. To understand irregularities in results obtained, at least 2 lots should be tested at each of the 3 testing sites. 10. The effect of operator-to-operator variation on IVD performance may also be considered as a human factor when designing robustness (flex) studies (see section 3.06.04a). The results of estimating operator-to-operator variation on IVD performance may be used in conjunction with studies to qualify the usability of the IVD. 11. Users shall be selected based on a pre-determined and contextually appropriate level of education, with	

IMDRF ToC chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
		literacy and auxiliary skills that will challenge the usability of the IVD and reflect the diversity of intended users (not laboratory professionals/technicians) and operational settings. These characteristics shall be detailed in the submission.	
3.05.05 Analytical sensitivity			
Limit of detection	1. Limit of Detection (LOD) shall be estimated relative to the International Standard(s) or to secondary standards metrologically traceable to it: • First WHO International Standard for *Plasmodium falciparum* antigens NIBSC code: 16/376. • First WHO International Standard for *Plasmodium vivax* antigen (LDH) NIBSC code: 19/116. 2. For claimed species and antigens where no international standard exists (that includes RDTs that detect Pf-LDH, and RDTs that detect Pf-HRP2/3 in combination with Pf-LDH), LOD shall be determined using a suitable biological reference material (see note 5). • At a minimum using specimens with P. falciparum parasites with HRP 2/3 deletions. 3. The determination of LOD shall comprise a minimum of 15—20 replicate tests of an 8-member dilution panel • In a clinical sample matrix.	1. The LOD shall be estimated by determining the lowest concentration for which the rate of detection is 95%. 2. For the international standard, the result shall be expressed in international units as an analytical end point sensitivity with its associated metrological uncertainty. 3. If the listed international standards are unavailable, the version of the international standard used shall be stated. 4. The LOD shall be sufficient to allow detection of a minimal clinically significant antigenemia, consistent with the median concentration of target antigen in the WHO Malaria rapid diagnostic test performance evaluation programme (see section A of this document). 5. Where a claim is made for "pan-specific" detection of *Plasmodium* species, LOD shall be estimated with WHO IS for *Plasmodium falciparum* antigens; WHO IS for *Plasmodium vivax* antigen (LDH); *Plasmodium falciparum* parasites with HRP2/3 deletions; and all other relevant species. • Note that specimens characterised as 'non-*P. falciparum*' are not sufficient.	PQDx_18 (*4*). ELISA SOPs (*6*) CLSI EP17-A2 (*12*) NIBSC (*15*)

IMDRF ToC chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	• Utilizing the entire assay system from sample preparation to interpretation. • Testing shall be conducted in 2 lots of the final locked-down design.	• If LOD is not determined in all relevant *Plasmodium* species, then this limitation of the IVD should be clearly reported as a warning to the user in the IFU. • Justification shall be provided for the choice of biological reference material used to determine the concentration of target antigen. The biological reference material used (where no IS exists), shall be characterized using ELISA to estimate the antigen concentration and corresponding LOD reported as antigen concentrations in pg/mL (see source document (***6***) for information on characterisation of specimens).	
3.05.06 Analytical specificity			
3.05.06a Potentially interfering substances and medical conditions	1. The potential for false results (falsely reactive and falsely non-reactive) arising from interference from, at least, the substances/conditions listed below shall be determined (see note 1). • Minimum of 100 specimens. • Each substance/condition is represented by at least 3—5 specimens from different individuals. 2. Testing shall be undertaken in *Plasmodium* spp. non-reactive and *Plasmodium* spp. reactive specimens (see note 4; unspiked or spiked), with each potentially interfering substance at high	1. The risk assessment conducted for an IVD should identify substances where the potential for interference can reasonably be expected for the analyte being detected (e.g. Pf-HRP2, pLDH, etc.). • By conducting and documenting appropriate risk assessment, testing can be performed on specimens spiked with the substances/ conditions identified as likely to be significant and testing of potentially irrelevant substances/conditions avoided.	CLSI EP07-A2 (***17***) CLSI EP37 (***18***) U.S. FDA Class II Special Controls Guidance document (***14***) ISO 14971:2019 (***19***) U.S. FDA Biotin guidance (***19***)

IMDRF ToC chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	physiologically relevant or medically relevant dosages (see note 3)	• Not by simple reliance on published lists of such compounds and conditions, which might be of limited relevance to this analyte. • Under some circumstances stringent risk evaluation might eliminate the necessity to test some of the items in the test requirements column (see paragraphs above) but any such decision shall be documented in the submissions to WHO and considered in the risk-benefit statements. • For RDTs that detect more than one plasmodium species, all plasmodium species should be included in testing, or a scientific justification provided if not. • Any effect must be evaluated against the probability of that effect occurring, given the prevalence of that substance in each of the populations intended to be tested and the clinical significance of the effect. 2. Any observed interference shall be investigated and performance limitations of the IVD reported in the instructions for use. Results shall be reported with respect to each condition and not be reported as an aggregate of the total number of specimens tested in the study. 3. The concentrations of the substances shall be provided 4. Interference studies should be performed with plasmodium specimens with a concentration near the LOD	
3.05.06b Endogenous substances	1. Human antibodies to the expression system (for recombinants), e.g., Anti-*Escherichia coli* (anti-*E.coli* positive), Human anti-mouse antibody (HAMA). 2. Recipients of multiple blood transfusions, pregnant (including multiparous) women. 3. Elevated levels of haemoglobin, lipids, bilirubin and protein. 4. Elevated IgG and IgM. 5. Rheumatoid factor. 6. Other autoimmune conditions.		
3.05.06c Exogenous substances	1. Relevant medicines, including: antiparasitic, antimalarial, antiretroviral and anti-tuberculosis medications. 2. Common over-the-counter anti-inflammatory medications (aspirin, paracetamol, ibuprofen). 3. Ethanol, caffeine.		

IMDRF ToC chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
		5. If the IVD detection mechanism employs streptavidin, then biotin levels of up to 3500 ng/mL should be tested as part of this study.	
3.05.06d Cross-reactivity	Determination of the potential for false results arising from cross-reactivity shall be investigated for a total of a minimum of 200 specimens, for at least 3—5 each of: 1. Viral infections, including HIV, hepatitis B, C infection, acute hepatitis A infection, dengue, yellow fever virus post-immunization, measles, influenza A and B, tick borne encephalitis, SARS-CoV-2. 2. Bacteria/parasites, including *Trypanosoma cruzi, Leishmania sp., Leptospira sp., Treponema pallidum, M. tuberculosis, Schistosoma sp., Toxoplasma gondii, Brucella sp.* 3. Other Plasmodium species (not claimed for detection by the RDT) e.g. *P.falciparum, P. vivax, P. ovale, and P. malariae.* 4. Other unrelated conditions known to cause cross-reactivity.	1. The potential cross-reacting organisms tested for should be risk-based, considering the operational setting and intended testing population. 2. Where either the scientific literature and/or risk analysis identifies the potential for false results in co-infected individuals (e.g. decreased sensitivity or specificity), further investigation shall be undertaken using *Plasmodium*-negative and -positive specimens. 3. Any observed interference shall be investigated and performance limitations of the IVD reported in the IFU. 4. Results shall be reported with respect to each condition and not be reported as an aggregate of the total number of specimens tested in the study. 5. For cross reactivity studies, where clinical specimens from individuals with the disease state to be tested are unavailable, a negative specimen shall be spiked with the organism of interest to a high concentration (a minimum of 10^5 plaque forming units/mL for viruses and 10^6 colony forming units/mL for bacteria). The use of recombinant antigen is not recommended.	
3.05.07 High dose hook effect			
High dose hook effect	The potential for a prozone/high dose hook effect shall be determined: 1. Using multiple, highly reactive natural specimens (minimum of 20).	1. Specimens shall be chosen that have a high analyte concentration, as determined using an test method other than the RDT intended to be prequalified. The	Butch, AW (*20*)

IMDRF ToC chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	2. Using at least 2 different concentrations (diluted by at least a factor of 10). 3. Using at least 1 lot of IVD.	second method shall be of a design not subject to competitive inhibition. 2. If there is evidence of competitive inhibition, this information shall be added to the IFU, and mitigation actions identified.	
3.05.10 Validation of the assay procedure			
Validation of the control line	1. The flow device shall have a control line. The nature of the control line shall be explained (see note 1, 2).	1. The extent to which any control line or dot corresponds to a valid test shall be validated. 2. The precise meaning of the control line must be stated in the IFU of the device, e.g. evidence of: • Reagent addition and flow. • Specimen addition and flow. • Correct volumes being added. • Correct operation of the device. • Correct functionality of all reagents.	
3.06 Other studies			
3.06.04 Usability/human factors			
3.06.04a Flex studies/ robustness	The influence of the following factors on expected results shall be considered (see notes 1 & 2): 1. Temperature. 2. Reading time (i.e., the interval between when the first and last readings may be taken). 3. Specimen and/or reagent volume. 4. Buffer pH.	1. Refer to WHO document PQDx_018 "Instructions for compilation of a product dossier" for other flex studies that may be relevant, taking into consideration the broad range of operational and environmental conditions consistent with intended use. 2. Testing should be undertaken using a panel consisting of: • 1 non-reactive specimen. • 1 low-reactivity specimen near assay LOD.	PQDx_018 (*4*).

IMDRF ToC chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	5. Buffer concentration (to account for evaporation, whether in single- or multiple-use containers). 6. Lighting and humidity.	• 1 medium-reactivity specimen. 3. The factors listed opposite should be investigated in ways that not only reflect but also exceed likely operating conditions in lower- and middle-income countries so that the limitations of the device to be understood. For example, in addition to investigating deviations of temperature within those claimed in the instructions for use (in the middle and at both lower and upper extremes of a claimed temperature range), temperature ranges should be investigated that exceed those of claimed operating conditions and which cause test failure (incorrect/invalid results). 4. If use of an IVD relies on particular operational conditions (e.g. temperature), these shall be reported in the IFU.	
3.06.04b Qualification of usability: label comprehension study	1. Questionnaire-based testing of subjects shall be undertaken to assess ability of intended users to correctly comprehend key messages from packaging and labelling: • Understanding key warnings, limitations and/or restrictions. • Proper test procedure. • Test result interpretation. 2. Questionnaires shall be administered to at least 15 intended users to demonstrate comprehension of key messages in each expected user described in note.3	1. Rpiequirements 1 and 2 may be investigated as separate studies or included as part of clinical studies 2. Instructions for use and labelling should be clear and easy to understand. Use of pictorial instructional material is encouraged. 3. Prequalified malaria RDTs will generally be used by trained lay providers and trained health care workers. For WHO prequalification purposes, these shall be considered as the intended user rather than a laboratory professional.	USAID and WHO (*21*) IEC 62366-1:2015 (***23***) Backinger CL and Kingsley PA (***24***)

IMDRF ToC chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
3.06.04 c Qualification of usability: results interpretation study	1. Subjects shall interpret the results of contrived IVDs (e.g. static/pre-made tests) to assess their ability to correctly interpret pre-determined test results. Contrived tests shall be made to demonstrate the following potential test results: • Non-reactive. • Range of invalid results. • Reactive. • Weak reactive. 2. Testing subjects shall consist of at least 15 intended users from 2 geographically diverse populations to demonstrate correct interpretation of simulated test results (see note 3).		
3.06.05 Stability			
3.06.05.01 & 3.06.05.03 Claimed shelf-life including transport stability	1. Real time studies shall be conducted using a minimum of 3 lots (see note 3). 2. The lots shall be transport stressed (simulated) before real time studies are undertaken (see note 6). 3. IVD in final packaging shall also be subjected to drop-shock testing. 4. Replicate testing shall be undertaken using a panel consisting, for each claimed analyte, of at least: • 1 analyte non-reactive specimen • 2 low-reactive specimens near assay LOD • 1 medium-reactive specimen. 5. Venous blood may be used.	1. The testing panel shall include all claimed antigens (e.g. Pf-HRP2/3, Pf-LDH, Pv-LDH etc.) and, where 'pan-specific' detection is claimed, address stability in all relevant *Plasmodium* species. 2. Testing shall include whole blood specimens in accordance with intended use (for example to verify proper flow, no background interference and account for other variables). 3. Lots shall comprise different batches of critical components. 4. Low-reactivity specimens shall be chosen that are sufficiently close to the assay LOD as to allow changes in IVD sensitivity to be detected.	ISO 23640:2011 (*25*) CLSI EP25 2nd edition (*26*) TGS-2 (*27*) Annex to TGS-2 (*28*) ASTM D4169-22 (*29*)

IMDRF ToC chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
3.06.05.02 In-use stability (open pack or open vial stability)	1. Minimum of 1 lot shall be tested using panel(s) compiled as above. 2. Testing of all labile components (e.g. buffers vials, sealed pouches etc.) shall be conducted. 3. In-use stability of labile components shall be conducted using components in their final configuration.	5. The numbers of invalid tests shall be reported. 6. Determination of shipping stability shall be performed using simulated extreme stress conditions, ensuring that application of those conditions is consistent and controlled. 7. Claims for stability shall be based on the second-last successful data point from the least stable lot, with, if lots are different, a statistical analysis showing that the bulk of lots will be expected to meet the claimed life. For example: for testing conducted at 3, 6, 9, 12 and 15 months, if stability was observed at 15 months, then the maximum stability claim is 12 months. 8. Accelerated studies do not replace the need for real time studies.	
3.08 Other evidence			
Performance panels	1. Testing of the IVD shall be against suitable performance panels (e.g. comprising relevant antigen variants, subtypes, etc.) where these are available. 2. Specimens that are *Plasmodium*-positive shall be correctly identified by the RDT.	1. Testing should be performed using more than 1 lot.	

Part 2 IMDRF ToC chapter 4 Clinical evidence

IMDRF ToC chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
4.02 Overall clinical evidence summary			
4.02.03 Device specific clinical studies			
4.02.03a General requirements for clinical evaluation studies	1. Clinical sensitivity and specificity shall be determined principally in capillary whole blood. 2. Testing should be conducted: • At different geographical and epidemiological settings representative of intended populations (minimum of 2 regions). • By a variety of intended users (i.e. 9—12 users). • Using more than 1 lot. 3. The number of specimens required below refer to the combined number of specimens collected for all specimen types and all geographical and epidemiological settings and regions.	1. Prequalified malaria RDTs will generally be used by trained lay providers and trained health care workers. For WHO prequalification purposes, these should also be considered as the intended user in addition to a laboratory professional. 2. A separate, venous whole blood specimen shall be collected in parallel to establish the reference result (using microscopy). The testing algorithm used to determine the reference results shall include microscopy and PCR (for identification/differentiation of species). Justification for the use of the testing algorithm and PCR test chosen shall be provided. 3. Lots (design locked down) shall comprise different batches of critical components. 4. The protocol shall specify the criteria for unbiased patient selection with associated risk analysis but in general there should be no exclusions except for ethical reasons. 5. All discrepant results (between assay under evaluation and the reference results) shall be repeated on the same lot, and then on all available lots and the variability noted. Performance characteristics shall be reported using initial results only. The results of further testing of specimens with discrepant results shall be	WHO Technical Report Series 366 (*30*) U.S. FDA (*31*) TGS-3 (*3*)
4.02.03b Clinical sensitivity	1. For RDTs intended for detection of *P. falciparum*: • At least 400 confirmed *P. falciparum*-positive specimens in total from a symptomatic population shall be tested. 2. For RDTs intended for detection of *P. vivax*: • At least 100 confirmed *P. vivax*-positive specimens in total shall be tested. 3. Where a claim is made for "pan-specific" detection of *Plasmodium* species, performance		

IMDRF ToC chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	characteristics shall be determined in each species for which specimens are available. At a minimum this shall include detection in specimens positive for *P. falciparum* and *P. vivax* (Note that specimens characterised as "non-*P. falciparum"* are not sufficient). Where testing in these specimens has not been undertaken, this limitation of IVD performance should be reported to the user as a warning in the instructions for use. 4. For RDTs detecting Pf- or pan-LDH antigen, prospective sampling of specimens with gene deletion(s) is required • 30 *Plasmodium falciparum* reactive specimens with HRP2/3 gene deletions shall be tested. At least 20 of these specimens shall include double deletions (both HRP2 and 3). • Testing is only required in 1 region.	reported separately as additional information about IVD performance. 6. All invalid results shall be recorded and evaluated in comparison to the reference result. Invalid results should be analyzed separately in the final performance calculations. 7. Estimates of diagnostic/clinical sensitivity and specificity shall be reported with 95% confidence intervals. 8. Results shall be reported with respect to each study site and not be reported as an aggregate of the total number of specimens tested to establish these characteristics. 9. A study protocol, dated and signed by authorized personnel, should be provided for each clinical evaluation study, including complete details about the patient selection, IVD characteristics, the reference test, and a description of the procedure. • lot numbers, product codes, IFU version etc. shall be provided in the study plan/report (see TGS-3).	
4.02.03c Clinical specificity	1. Testing shall be conducted of: • At least 1000 *Plasmodium* negative specimens in total from a symptomatic population.		

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