TSS-3

Malaria rapid diagnostic tests second edition, draft for comment

Technical specifications series for submission to WHO prequalification – diagnostic assessment

World Health Organization

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Contents

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Meeting participants: Michael Aidoo, Center for Disease Control and Prevention, Atlanta, United States of America (USA); Paul Bowyer, Medicines and Healthcare products Regulatory Agency (MHRA), London, United Kingdom of Great Britain and Northern Ireland; Eleanor Brindle, PATH, Seattle, USA; Qin Cheng, Australian Defence Force Malaria & Infectious Disease Institute Laboratory, Australia; Elizabeth George, MRC Clinical Trials Unit, University College London, United Kingdom[1]; Allison Golden, PATH, Seattle, USA, Marcos V. Guimaraes De Lacerda, Instituto Leônidas & Maria Deane, Fiocruz, Manaus, Brazil; Liselotte Hardy, Institute of Tropical Medicine Antwerp, Belgium; Rosalind Howes, FIND, Geneva, Switzerland; Christine Pfeiffer, Center for Disease Control and Prevention, Atlanta, USA; Kamija Phiri, University of Malawi, Blantyre, Malawi; Erwan Piriou, Médecins Sans Frontières, Amsterdam, The Netherlands (the Kingdom of); Scott Ruetten, Independent consultant, USA[2]; Johannes Schweizer, Independent consultant, USA; James Tibenderana, Malaria Consortium, London, United Kingdom.

WHO Secretariat: Charles Chiku; Jean-Frédéric Flandin; Deirdre Healy; Mark Lanigan; Anne-Laure Page; Irena Prat, Ute Ströher, In Vitro Diagnostics Assessment Team, Regulation and Prequalification Department; Lisa Rogers and Maria Nieves Garcia-Casal, Food and Nutrition Actions in Health Systems Unit (AHS); Jane Cunningham, Emerging diseases and zoonoses; Andrea Bosman, Global malaria programme. [3]

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[1] Via teleconference
[2] Via teleconference
[3] Via teleconference

Shanghai Kehua Bio-engineering Co., Ltd, Shanghai, China; Standard Diagnostics, Inc, Gyeonggi-do, Korea.

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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

Abbreviations

ANOVA	analysis of variance
HAMA	human anti-mouse antibody
HRP2/3	histidine-rich protein 2/3
IFU	instructions for use
IgG, IgM	immunoglobulins G and M
IMDRF ToC	International Medical Device Regulators Forum "Table of Contents"
IVD	in vitro diagnostic medical device
PCR	polymerase chain reaction
pfHRP2	*Plasmodium falciparum* Histidine-Rich Protein 2
pLDH	plasmodium lactate dehydrogenase
RDTs	rapid diagnostic tests
TGS	Technical Guidance Series
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 to 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 p/µL) from the product testing evaluation panel for the detection of P. *falciparum* (HRP2 (Histidine Rich Protein 2) 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 to demonstrate that reasonable steps have been taken to ensure that a properly manufactured IVD, being correctly operated in the hands of the intended user, will detect the target analyte and fulfil its indications for use.

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 for WHO prequalification – diagnostic assessment available (*3*)
- Instructions for compilation of a product dossier, WHO document PQDx_018. (*4*).

WHO Global Malaria Programme documents:

- Quality and safety practices for malaria rapid testing services (5)
- Methods manual for laboratory quality control testing of malaria RDTs (6)
- False-negative RDT results and implications of new reports of P. falciparum histidine-rich protein 2/3 gene deletions. (7)

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;
- What the IVD measures or detects: (e.g. to detect P. falciparum LDH antigen; pan-specific detection of all Plasmodium species; detection of, and differentiation between, Plasmodium and non-Plasmodium species);
- The function of the IVD; (e.g., assist in the diagnosis of malaria by detecting evidence of malaria parasites (antigens) in human blood);
- 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);
- What the IVD reports;
- The intended use environment;
- The intended user;
- 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[4] and at point-of-care by healthcare workers or trained lay providers[5]. 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 summarized 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 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 normal human specimens have been spiked with *Plasmodium* reactive specimens) should only be used in the submitted studies if a 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; additional supporting evidence may also be required. This may occur following minor variations to design where no negative impact on performance has been demonstrated (see WHO document Reportable Changes to a WHO prequalified in vitro diagnostic medical device. (***8***)

For IVDs 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. IVDs claiming to provide 'pan'-specific detection of malaria are expected to detect all known pathogenic species of *Plasmodium*, i.e.: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. Full characterisation of species is required including quantification by ELISA (***6***)

[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.

and reporting should clearly state results for each species. If performance characteristics are not established for all relevant species of *Plasmodium* in IVDs claiming detection of "pan"-specific detection, this limitation of the IVD shall be clearly reported as a warning to the user in the instructions for use.

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* Histidine-rich protein 2 (PfHRP2) detection in a PfHRP2-only IVD will not be accepted as evidence to support PfHRP2 detection in a subsequent dual-detection version of the IVD designed to detect both PfHRP2 and *Plasmodium* lactate dehydrogenase (pLDH).

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:2019 (Edition 3). (*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 specimens(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.	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			
3.05.02 a 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 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.*	TGS-3 (*10*)
3.05.02 b 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).		

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	2. Equivalence shall be determined for each claimed *Plasmodium* antigen.	• *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. 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:	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. PfHRP2, pLDH, etc., as appropriate). 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.	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
	• 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 personnel representative of intended users (note 11). • Unassisted. • Using only those materials provided with the IVD (e.g. instructions for use, labels and other instructional materials).	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 Flex studies). 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 literacy and auxiliary skills that will challenge the usability of the IVD and reflect the diversity of intended users and operational settings. These characteristics shall be detailed in the submission.	

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
3.05.05 Analytical sensitivity			
Limit of Detection	1. Limit of Detection (LOD) shall be estimated relative to the international standards 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, LOD shall be determined using a suitable biological reference material. • 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. • Utilizing the entire assay system from sample preparation to interpretation. • Testing shall be conducted in 2 lots of the final locked-down design.	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. • 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	PQDx_18 (*1*) ELISA SOPs (*4*) CLSI EP17-A2 (*11*) NIBSC (*15*)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
		be characterized using ELISA to estimate the antigen concentration and corresponding LOD reported as antigens/µL (see source document (*4*) for information on characterisation of specimens).	
3.05.06 Analytical specificity			
3.05.06 a 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 both *Plasmodium*-negative and -positive specimens unspiked or spiked with each potentially interfering substance at physiologically relevant dosages.	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. PfHRP2, 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. • 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. • Any effect must be evaluated against the probability of that effect occurring, given the prevalence of that substance in each of the	EU Common specifications (*15*) CLSI EP07-A2 (*17*) CLSI EP37 (*18*) FDA Class II Special Controls Guidance document, FDA (*14*) ISO 14971:2019(*19*)
3.05.06 b 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.		

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	6. Other autoimmune conditions.	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.	
3.05.06 c 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.		
3.05.06 d 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 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 users. 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	

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
		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). 2. Using at least 2 different concentrations (diluted by at least a factor of 10). 3. Using at least 1 lot of IVD.	1. Specimens shall be chosen that have a high analyte concentration, as determined using an IVD method other than the RDT intended to be prequalified. The 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.	Butch, AW (*20*)
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.	
3.06.04 Usability/human factors			
3.06.04 a Flex studies/ robustness	The influence of the following factors on expected results shall be considered: 1. Temperature (see notes 1 & 2).	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	PQDx_018 (*1*)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	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. 5. Buffer concentration (to account for evaporation, whether in single- or multiple-use containers). 6. Lighting and humidity.	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 specimens near assay LOD. • 1 medium-reactivity specimen. 3. If use of an IVD relies on particular operational conditions (e.g. temperature), these shall be reported in the IFU. 4. 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).	
3.06.04 b 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.	1. Requirements listed opposite 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	USAID and WHO (*20*) EU IVDR (*22*) IEC 62366-1:2015 (*22*)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	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.	as the intended user rather than a laboratory professional.	Backinger CL and Kingsley PA (*24*)
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).		

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
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 of final design product. 2. The lots shall be transport stressed (simulated) before real time studies are undertaken. 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-reactivity specimens near assay LOD • 1 medium-reactivity specimen. 5. Venous blood may be used.	1. The testing panel shall include all claimed antigens (e.g. PfHRP2, pLDH, etc.) and, where 'pan-specific' detection is claimed, address stability in 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. 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.	ISO 23640:2011 (*25*) CLSI EP25 2nd edition (*26*) TGS-2 (*27*) Annex to TGS-2 (*28*) ASTM D4169-22 (*29*)
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 cartridges, etc.; see note 9) shall be conducted. 3. In-use stability of labile components shall be conducted using components in their final configuration.		

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
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 IVD.	1. Testing should be performed using more than one lot of the final design (locked-down).	PQDx_018 (*1*)

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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			
4.02.03 a General requirements for clinical evaluation studies	1. Diagnostic 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 users (minimum of 2 regions). • By a variety of intended users (i.e. 9 - 12 users). • Using more than 1 lot.	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 differentiation of species). Justification for the use of the testing algorithm shall be provided. 3. Lots (design locked down) shall comprise different batches of critical components. 4. 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 reported separately as additional information about IVD performance. 5. All invalid results shall be recorded and evaluated in comparison to the reference result. Invalid results	EU IVDR (*22*) WHO Technical Report Series 366 (*30*) U.S. FDA (*31*)
4.02.03 b Diagnostic sensitivity	1. For IVDs intended for detection of *P. falciparum*: • At least 400 confirmed *P. falciparum*-positive specimens from a symptomatic population shall be tested. 2. For IVDs intended for detection of *P. vivax*: • At least 100 confirmed *P. vivax*-positive specimens shall be tested. 3. Where a claim is made for "pan-specific" detection of *Plasmodium* species, performance 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.*		

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	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 LDH antigen, prospective sampling of gene deletion specimens is required • 30 specimens with HRP2/3 gene deletions shall be tested. At least 20 of these specimens shall include double deletions (both HRP2 and 3). • Testing in 1 region only is required.	should be analyzed separately in the final performance calculations. 6. Estimates of diagnostic/clinical sensitivity and specificity shall be reported with 95% confidence intervals. 7. Where an IVD is intended to detect multiple analytes without differentiating which analyte is detected, specimens chosen for the testing panel shall comprise those that are reactive only for each individual analyte (i.e. PfHRP2, pLDH, etc., as appropriate). 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.	
4.02.03 c Diagnostic specificity	1. Testing shall be conducted of: • At least 1000 *Plasmodium* negative specimens from a symptomatic population.		

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Source documents

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