Technical Specifications Series for submission to WHO Prequalification – Diagnostic Assessment

TSS-3 [Draft] Malaria rapid diagnostic tests

Draft for comment, version 05 December 2016
# Technical Specifications for submission to WHO Prequalification – Diagnostic Assessment: [Draft] Malaria rapid diagnostic tests

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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 (PQ) of IVDs for the detection, in blood, of antigens produced by Plasmodium (malaria) species. For the purposes of PQ, this document applies only to RDTs intended to diagnose malaria infection.

Minimum performance requirements for PQ are summarized herein and, where possible, are aligned with published guidance, standards and/or regulatory documents. Although references to source documents are provided, in some cases WHO PQ may have additional requirements.

For PQ purposes, manufacturers must provide evidence in support of the clinical performance of an IVD which can 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.

PQ 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 PQ.

B. Other guidance documents

It is recommended that this document is read in conjunction with other WHO guidance documentation, including:

- Technical Guidance Series (TGS)
- Sample Product Dossiers for WHO Prequalification
- WHO document PQDx_018 Instructions for Compilation of a Product Dossier.

These documents are available at: http://www.who.int/diagnostics_laboratory/evaluations/en/

C. Performance Principles for WHO Prequalification

C.1 Intended use

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

- The function of the IVD (e.g. to detect *P. falciparum* infection; pan-specific detection of all *Plasmodium* species; detection of, and differentiation between, *Plasmodium* and non-*Plasmodium* species),
- The testing population for which functions are intended (e.g. neonatal screening), and
- Clinical indication (e.g. to diagnose malaria infection).

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

For WHO purposes, clinical performance studies should be conducted using the specimen types most likely to be used in resource-limited WHO Member States (e.g. capillary whole blood from children and/or neonates). If this is not possible, then 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\(^1\) and at point-of-care by healthcare workers or trained lay providers\(^2\). Depending on the intended use of an IVD, performance studies must be designed to take into account not only the diversity of knowledge and skills across the population of IVD users, but also the likely operational settings in which testing will occur. For example, studies that comprise the testing of leftover/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 requirement summarised 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.

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\(^1\) Medical technologists, medical laboratory technicians or similar, who have received a formal professional or paraprofessional certificate or tertiary education degree.

\(^2\) 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 certificate or tertiary education degree.
C.3 Applicability of supporting evidence to IVD under review

The true *Plasmodium* status of a specimen must be determined using a suitable reference method, justification for which must be provided.

Estimation (and reporting) of IVD performance must include the rate of invalid test results (where ‘invalid’ is a result interpretation defined in the IFU).

For certain analytical studies, it may be acceptable to use contrived specimens (e.g. where normal human specimens have been spiked with *Plasmodium* reactive specimens). Although all reasonable attempts should be made to use natural specimens, justification should be provided where contrived specimens are used in the submitted studies. The use of recombinant *Plasmodium* antigens should be avoided. Clinical studies should be based on testing in natural specimens only.

Studies should be undertaken using the final, or locked-down version of the IVD that is intended to be prequalified. Where this is not possible (e.g. because of design variation) a justification must be provided; additional supporting evidence may also be required. This can occur as minor variations to design where no negative impact on performance has been demonstrated.

For IVDs that include a claim for detection of multiple antigens and/or species, evidence of performance must be provided for each claimed antigen and/or species. For the purposes of WHO PQ IVDs claiming ‘pan’-specific detection of malaria are expected, where possible, to detect at least: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. Where a claim is made for ‘pan-specific’ detection of *Plasmodium* species, every effort should be made to determine performance characteristics in each species for which specimens are available. At a minimum this should include detection in specimens positive for *P. falciparum* and *P. vivax* (NB: specimens characterised as ‘non-*P. falciparum*’ are not sufficient). For performance characteristics not investigated in all relevant species of *Plasmodium*, this limitation of the IVD should be clearly reported as a warning to the user in the IFU.

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 prequalification. For example, evidence of 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 pLDH.
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## Part 1  Establishing analytical performance

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| 1.1 Specimen type | For each claimed specimen type, testing in at least:  
  - 25 *Plasmodium* negative; and  
  - 25 *Plasmodium* positive specimens.  
Equivalence must be determined for each claimed *Plasmodium* antigen and/or species, as appropriate.  
Ideally, positive specimens should be chosen so that the majority are near the IVD cut-off. | 1. The relationship should be established between claimed specimen types and reference materials for analytical studies. The design of subsequent studies must then take that relationship into account. If there is no equivalence between claimed specimen types then the impact that this will have on each subsequent performance claim must 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 must be understood.  
- This might 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). | WHO TGS-3 [1] |
| 1.1.1 Demonstration of equivalence between specimen types | | | |
| 1.1.2 Demonstration of equivalence of claimed anticoagulants | For each claimed anticoagulant, testing in at least:  
  - 25 *Plasmodium* negative; and  
  - 25 *Plasmodium* positive specimens.  
Equivalence must be determined for each claimed *Plasmodium* antigen.  
Positive specimens must be chosen so that a majority are near the cut-off. | | |
| 1.2 Specimen collection, storage and transport | Real time studies taking into account:  
  - Storage conditions (duration at different temperatures and variation in humidity, temperature limits, freeze/thaw cycles). | 1. Particular attention should be paid to the length of time likely to elapse between specimen collection and its addition to the IVD in the setting this IVD may be used. | WHO TGS-1 [2] |
| 1.2.1 Specimen stability | | | |
Technical Specifications for WHO Prequalification of malaria RDTs

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| • Transport conditions, where applicable.  
• Intended use (see comment 1).  
• Specimen collection and/or transfer devices, whether these contain anticoagulants and whether they can be sealed. | | | |

### 1.3 Precision of measurement

#### 1.3.1 Repeatability, reproducibility

Both repeatability (within-condition – see comment 1) and reproducibility (between-condition – see comment 1) estimated by replicate testing of end-point dilutions of several analyte-positive specimens.

Specimens chosen for the testing panel must include panel members that reflect the main specimen types intended for use with the IVD (e.g. capillary whole blood).

Each panel member tested:

- using 3 different lots (see Comment 9);
- over 5 days (not necessarily consecutive) with one run in that day (alternating morning/afternoon);
- at each of 3 different testing sites; and
- at two temperatures within the claimed operating range [for enzymatic IVDs].

The effect of operator-to-operator variation on IVD performance is to be included as part of the precision studies (see also Comment 10). Testing should be performed:

- by personnel representative of intended users;
- unassisted; and
- using only those materials provided with the IVD (e.g. IFU, labels and other instructional materials).

1. E.g. within- or between-run, -lot, -day, -operator, -site, etc.
2. Precision must 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 should be chosen that are sufficiently close to the assay cut-off as to allow changes in IVD sensitivity to be detected.
5. The numbers of invalid tests must be reported.
6. Lots must be composed of different batches of critical components.
7. Results must be statistically analyzed (e.g. using ANOVA 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 should 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 which may not have any numerical values for ANOVA analysis.
9. To understand irregularities in results obtained it is recommended that at least 2 lots are tested at each of the 3 testing sites.
10. The effect of operator-to-operator variation on IVD performance is also to be considered as a human factor when designing robustness (flex) studies (see 1.10.1 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

CLSI EP05-A3 [3]
Technical Specifications for WHO Prequalification of malaria RDTs

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<td>1.4 Performance panels</td>
<td>Testing of the IVD in suitable performance panels (e.g. comprising relevant antigen variants, subtypes, etc.) where these are available.</td>
<td>Specimens that are <em>Plasmodium</em>-positive must be correctly identified by the IVD.</td>
<td>WHO [6]</td>
</tr>
<tr>
<td>1.5 Validation of reading time</td>
<td>For IVDs where a reading interval is specified (i.e., time when result can first be read; time beyond which result should not be read) validation of critical time points must be provided. Performance studies should be conducted at intended operational temperature; the effect of humidity on reading times should also be investigated.</td>
<td>The ranges of humidity tested for should be risk-based, taking into consideration likely operational settings.</td>
<td>TGS-3 [1]</td>
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### 1.6 Analytical sensitivity

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<td>1.6.1 Analytical Sensitivity</td>
<td>Analytical sensitivity may be determined by testing in at least 8-member dilution series. At least 15-20 replicates tested per dilution. Analytical sensitivity estimated by determining the lowest concentration for which the rate of detection is 95%. Where a claim is made for ‘pan-specific’ detection of <em>Plasmodium</em> species, every effort should be made to determine analytical sensitivity in each species for which specimens are available. At a minimum this should include detection in specimens positive for <em>P. falciparum</em> and <em>P. vivax</em> (NB: specimens characterised as “non – <em>P. falciparum</em>” are not sufficient). If analytical sensitivity is not detected in all relevant species of <em>Plasmodium</em> species, then this limitation of the IVD should be clearly reported as a warning to the user in the IFU.</td>
<td>1. Analytical sensitivity should be determined for each claimed antigen (e.g. PfHRP2, pLDH, etc.) and/or species, as appropriate. 2. Analytical sensitivity should be at least 100 parasites/μl. 3. Antigen detection must be investigated using specimens that represent relevant stages of parasite development. 4. Optimally, testing should be conducted using more than one final design (locked-down) lot. 5. Analytical sensitivity should be demonstrated in clinical sample matrix and should use the entire assay system from sample preparation to interpretation. 6. It is recommended that the estimate of analytical sensitivity be confirmed by separately testing an additional 20 replicates.</td>
<td>CLSI EP17-A2 [4] FDA [7] WHO [6]</td>
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<td>1.6.2 Validation of assay cut-off</td>
<td>Malaria RDTs are generally qualitative and do not use a numerical value of assay cut-off. Nevertheless the way in which the IVD was designed to differentiate positive specimens from negative specimens should be demonstrated.</td>
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<td>1.6.3 Measuring range</td>
<td>The potential for a prozone/high-dose hook effect should be determined using several <em>Plasmodium</em>-positive specimens, for each claimed antigen, tested in several replicates at two different concentrations (dilated by at least a factor of 10).</td>
<td>1. Specimens should be chosen that have a high antigen concentration, as estimated using microscopy or PCR.</td>
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### 1.7 Analytical specificity

#### 1.7.1 Potentially interfering substances

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|                         | The potential for false results (false negatives and false positives) arising from interference for at least the substances/conditions listed below should be determined. | 1. The risk assessment conducted for an IVD should identify substances for which the potential for interference is reasonably foreseeable.  
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 should be undertaken using Plasmodium-negative and -positive specimens.  
3. Any observed interference should be investigated and performance limitations of the IVD reported in the IFU. Results should be reported with respect to each condition and not be reported as an aggregate of the total number of specimens tested in the study. | EU CTS [8]  
CLSI EP07-A2 [9]  
FDA [7] |
| Endogenous              | - Human antibodies to the expression system (for recombinants), e.g., Anti-\textit{Escherichia coli} (anti-E.coli positive), Human anti-mouse antibody (HAMA).  
- Recipients of multiple blood transfusions, 200 pregnant (including multiparous) women.  
- Elevated levels of haemoglobin, lipids, bilirubin and protein.  
- Elevated IgG and IgM.  
- Rheumatoid factor.  
- Other autoimmune conditions. |                                                                 |                     |
| Exogenous               | - Relevant medicines, including: antiparasitic, antimalarial, antiretroviral and anti-tuberculosis medications.  
- Common over-the-counter anti-inflammatory medications (aspirin, paracetamol, ibuprofen).  
- Ethanol, caffeine. |                                                                 |                     |
1.7.2 **Cross-reactivity**

Determination of the potential for false results arising from cross-reactivity for a total of a minimum of 200 specimens, for at least 3-5 each of:

- Viral infections, including: HIV, HCV, HBV, acute HAV, dengue, yellow fever virus post-immunization, measles, influenza A and B, tick borne encephalitis.
- Bacteria/parasites, including: *Trypanosoma cruzi*, *Leishmania* sp., *Leptospira* sp., *Treponema pallidum*, *M. tuberculosis*, *Schistosoma* sp., *Toxoplasma gondii*, *Brucella* sp.
- Other unrelated conditions.

1. **The types of interferences tested for should be risk-based, taking into consideration the operational setting as well as the intended users.**
2. **Any observed interference should be investigated and performance limitations of the IVD reported in the IFU.**

1.8 **Metrological traceability of calibrators and control material values**

1.8.1 **Metrological traceability of calibrators and control material values**

The traceability of an external control to a validated reference material must be demonstrated.

1. **WHO encourages the use of external / quality control specimens which must be traceable to a validated reference material and demonstrate whether a test result is valid. Where malaria RDT uses a procedural control either in addition to, or instead of, an external control, the extent to which the presence or absence of this band corresponds to a valid test should be demonstrated.**

1.9 **Stability**

1.9.1 **IVD stability**

Replicate testing of a panel consisting, for each claimed analyte, of at least:

- 1 analyte non-reactive specimen;
- 2 low-reactivity specimens (near assay cut-off, e.g. approximately 100 parasites/µl) and
- 1 medium-reactivity specimen (e.g. approximately 1000 parasites/µl).

Where possible, specimens chosen for the testing panel must include panel members that reflect the main specimen types intended for use with the IVD (e.g. *Plasmodium* species).

1. **The testing panel must include all claimed antigens (e.g. PfHRP2, pLDH, etc.) and, where ‘pan-specific’ detection is claimed, address stability in relevant *Plasmodium* species.**
2. **Testing should 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 must comprise different batches of critical components.**
4. **Low-reactivity specimens should be chosen that are sufficiently close to the assay cut-off as to allow changes in IVD sensitivity to be demonstrated.**

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ISO 23640:2011 [10]
WHO TGS-2 [12]
ASTM D4169-14 [13]
5. The numbers of invalid tests must be reported.
6. Determination of shipping stability must be performed using simulated extreme stress conditions, ensuring that application of those conditions is consistent and controlled.
7. Claims for stability must 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 can be 12 months.
8. Accelerated studies do not replace the need for real time studies.
9. In-use stability of labile components should be conducted using components in their final configuration.

### Shelf life

- Real time, minimum of 3 lots of final design product
- Transport stressed (simulated) before real time studies are undertaken.
- IVD in final packaging also subjected to drop-shock testing.

### In-use stability

- Minimum of 1 lot, using panel(s) compiled as above.
- Testing of all labile components (e.g. buffers vials, sealed cartridges, etc.; see Comment 9).

### Flex studies

1. Refer to WHO document PQDx_018 “Instructions for compilation of a product dossier” [REF6] for other flex studies that may be relevant, taking into consideration the broad range of operational and environmental conditions consistent with intended use. If use of an IVD relies on particular operational conditions (e.g. temperature), these must be reported in the IFU.

WHO [6]
### Part 2 Establishing clinical performance

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| **2.1 Diagnostic sensitivity and specificity** | Diagnostic sensitivity and specificity should be determined principally in capillary whole blood. Testing should be conducted:  
- at different geographical settings representative of intended users (minimum of 2 regions);  
- by a variety of intended users (i.e. 9 - 12 users); and  
- using more than one lot. | 1. Prequalified malaria RDTs will generally be used by trained lay providers and trained health care workers. For prequalification purposes, these should be considered as the intended user, rather than a laboratory professional.  
2. A separate, venous whole blood specimen should be collected in parallel to establish the reference result. The testing algorithm used to determine the reference results should include should include microscopy and PCR. Justification for the use of the testing algorithm must be provided.  
3. Lots (design locked-down) must comprise different batches of critical components.  
4. All discrepant results (between assay under evaluation and the reference results) should be repeated on the same lot, and then on all available lots and the variability noted. Performance characteristics must be reported using initial results, only. The results of further testing of specimens with discrepant results must be reported separately as additional information about IVD performance.  
5. All invalid results must be recorded and evaluated in comparison to the reference result. Invalid results should be analyzed separately in the final performance calculations.  
6. Estimates of diagnostic/clinical sensitivity and specificity should be reported with two-sided 95% confidence intervals. | EU IVD [17]  
WHO [14]  
FDA [15] |
| **2.1.1 Diagnostic sensitivity and specificity** | Diagnostic Sensitivity:  
For IVDs intended for detection of P. falciparum:  
- At least 400 confirmed P. falciparum-positive specimens from a symptomatic population.  
For IVDs intended for detection of P. vivax:  
- At least 100 confirmed P. vivax-positive specimens.  
For other IVDs intended for detection of non-P. falciparum species (e.g. 'pan-specific' detection of pLDH) every effort should be made to test specimens containing P. malariae, P. ovale and/or P. knowlesi, as appropriate. 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 IFU. |  |  |
| | Diagnostic Specificity:  
- At least 1000 Plasmodium negative specimens from a symptomatic population. | | |
### 2.2 Qualification of usability

#### 2.2.1 Label comprehension study

Questionnaire-based testing of subjects 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; and
- test result interpretation.

Questionnaire to be administered to at least 200 intended users, in order to demonstrate comprehension of key messages in each population described above.

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<td>Should be clear and easy to understand; use of pictorial instructional material is encouraged. [REF 16]</td>
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<tr>
<td>2. Prequalified malaria RDTs</td>
<td>Will generally be used by trained lay providers and trained health care workers. For prequalification purposes, these should be considered as the intended user, rather than a laboratory professional.</td>
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#### 2.2.2 Results interpretation study

Subjects to 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 should be made to demonstrate the following potential test results:
- non-reactive;
- range of invalid results;
- reactive; and
- weak reactive.

Testing subjects to consist of at least 200 intended users from two geographically diverse populations to demonstrate correct interpretation of simulated test results.

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<tr>
<td>ISO 62366-1</td>
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<td>FDA</td>
<td>[19]</td>
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References


