TSS-22

Haemoglobin point of care analysers, 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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List of contributors

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Declarations of interests

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.

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

Abbreviations

Hb	haemoglobin
HiCn	cyanmethemoglobin
ICSH	International Committee for Standardization in Haematology
IFU	instructions for use
IMDRF ToC	International Medical Device Regulators Forum “Table of Contents”
IVD	in vitro diagnostic
LLOQ/ULOQ	lower/upper limit of quantification
LOB	limit of blank
LOQ	limit of quantification
LOD	limit of detection
MHRA	Medicines and Healthcare products Regulatory Agency, United Kingdom of Great Britain and Northern Ireland
NORAD	Norwegian Agency for Development Cooperation
POC	point-of-care
TSS	Technical specifications series
WHO	World Health Organization

A. Introduction

The purpose of this document is to provide technical guidance to in vitro diagnostic (IVD) medical device manufacturers that intend to seek WHO prequalification for point of care (POC)[4] IVDs for the quantitative detection of haemoglobin in capillary or venous whole blood.

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 suggested methods to undertake the testing, but 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.

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 consistently and fulfil its indications for use. The clinical performance study described in part 2 is intended to verify the performance of the IVD in the intended user and use setting. It is not intended to set diagnostic or treatment thresholds.

The 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 WHO guidance documents

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

- Technical guidance series documents for WHO prequalification - diagnostic assessment (*1*)

[4] Point of care in vitro diagnostic testing (POC) refers to decentralized testing that is performed by a minimally trained healthcare professional near a patient and outside of central laboratory testing facilities. It does not refer just to sample collection procedures. In some jurisdictions, the concept "near patient testing" is used instead of "point of care testing". Either term may be used in the intended use statement.

- Instructions for Compilation of a product dossier, WHO document PQDx_018 *(2)*
- Guideline on haemoglobin cutoffs to define anaemia in individuals and populations (*3*)

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 type of assay and what the IVD measures (POC assay to quantify haemoglobin levels in whole blood);
- The function of the IVD (e.g. screening for anaemia, monitoring of haemoglobin levels; diagnosis of anaemia/aid in the diagnosis of anaemia taking into account clinical signs)
- The specific disorder, condition or risk factor of interest that is intended to detect, define or differentiate (anaemia);
- Whether or not it includes automated components or is intended to be used with automated instruments;
- What the IVD reports (e.g. total haemoglobin in blood in g/dL, mg/dL or mmol/dL);
- The target population (e.g., all sections of the population)
- The intended use environment (e.g. POC setting, laboratory setting);
- The intended user (trained healthcare worker/lay provider[5], trained healthcare professional, laboratory professionals[6]);
- The intended specimen type (e.g. capillary blood drops or venous blood), including specimen source, matrix, time of sample collection and collection methods;
- Any limitations to the intended use or conditions that affect the test result;

Hb reference ranges in venous blood, according to WHO (*3*), are listed in Table 1. The values in the table below are intended to indicate to manufacturers the range of values to be tested in the analytical performance studies described in part 1 below. It is recognized that reference ranges cited in literature and guidances differ and that Hb concentration in blood can vary due to various factors (e.g. altitude, smoking, population groups, geographical regions). Furthermore, both the method of haemoglobin measurement and blood sample source (capillary versus venous blood) can affect the measured haemoglobin concentration. In addition, Hb levels tend to be lower in certain populations possibly due to poor nutritional status resulting in iron defiency (low levels of iron uptake), genetic disorders (e.g. thalassemia, sickle cell trait), or infection with helminths (causing chronic blood loss) or other parasites (e.g. malaria or schistosomiasis) (*4*). This should be taken into account when interpreting the Hb results of any POC IVD.

[5] Any person who performs functions related to healthcare delivery and has not received a formal professional or paraprofessional certification or tertiary education degree.

[6] Medical technologists, medical laboratory technicians or similar, who have received a formal professional or paraprofessional certification or tertiary education degree

Table 1: Haemoglobin cutoffs to define anaemia severity in individuals (***3***)

Population	Haemoglobin concentration (g/L) No anaemia	Mild anaemia	Moderate anaemia	Severe anaemia
Children 6-23 months	≥ 105	95-104	70-94	< 70
Children 24-59 months	≥ 110	100-109	70-99	< 70
Children 5-11 years	≥ 115	110-114	80-109	< 80
Children 12-14 years, nonpregnant girls	≥ 120	110-119	80-109	< 80
Children 12-14 years, boys	≥ 120	110-119	80-109	< 80
Adults 15-65 years nonpregnant women	≥ 120	110-119	80-109	< 80
Adults 15-65 years, men	≥ 130	110-129	80-109	< 80
Pregnancy				
First trimester	≥ 110	100-109	70-99	< 70
Second trimester	≥ 105	95-104	74-94	< 70
Third trimester	≥ 110	100-109	70-99	< 70

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

For WHO prequalification submission, clinical performance studies shall be conducted using each specimen type (e.g. capillary whole blood, venous whole blood) claimed in the instructions for use (IFU).

Prequalified Hb POC IVDs in low- and middle-income countries are likely to be used by a range of users in different geographical settings:

- Healthcare or laboratory professionals either in centralised testing laboratories or at POC,
- Healthcare or laboratory professionals in health care settings or at POC who are not experienced in biochemical testing,
- Lay providers trained in the use of the test at POC.

Depending on the intended use of the IVD, analytical and clinical performance studies shall be designed to take into account not only the diversity of knowledge and skills across the population of individuals using the IVD, intended use population, but also the likely operational settings (e.g. varying altitudes) in which testing will occur.

It is a manufacturer's responsibility to ensure that the risk assessment for an IVD reflects the intended environment of use and intended operational settings, including laboratory or service delivery complexity, user expertise, training received, test population, concomittent infections/medication.

C.3 Applicability of supporting evidence to IVD under review

Performance shall be established in comparison to the cyanmethemoglobin (HiCn) reference method/standard. Bias and uncertainty of the reference method shall be provided in the dossier and bias shall also be verified during the evaluation of the Hb IVD.

A quantitative comparator test may be acceptable as an alternative to the HiCn reference method if a justification is provided. The justification shall include metrological traceability

to the reference methodology (HiCn method). The comparator test shall be authorized for use by a recognized stringent regulatory authority[7] and acknowledged in the literature as representing state of the art. Determination of Hb levels using a quantitative comparator test shall take into account the imprecision and bias inherent in that test. At a minimum, all results of the comparator test obtained with reference materials e.g., JCCRM 912 Certified Reference Material for Total Hemoglobin Measurement from Reference Material Institute for Clinical Chemistry Standards (ReCCS), Japan) shall be within the following desirable analytical performance specifications based on biological variation (https://biologicalvariation.eu/) (*5*):

- bias ⩽1.7%
- imprecision ⩽1.4%
- total error ⩽3.9%

The corresponding quantitative values of Hb concentration (g/L, g/dL, mmol/dL), and how these were calculated shall be reported.

Analytical and clinical performance studies shall be undertaken using the specific, final (locked-down) version of the IVD intended to be submitted for WHO prequalification. For WHO prequalification, design lock-down is the date that final documentation, including quality control and quality assurance specifications, is signed off and the finalized method is stated in the IFU. Where this is not possible, a justification shall be provided, and additional supporting evidence may also be required. This may occur in the case of minor variations to design where no impact on performance has been demonstrated (see WHO document PQDx_121 Reportable Changes to a WHO Prequalified In Vitro Diagnostic Medical Device (*6*)).

If the method section of the IFU has been changed in any way, both the study protocol provided to the laboratory and that in the final version of the IFU intended for users shall be provided with the submission for WHO prequalification assessment. The version of the IFU used in the verification and validation studies submitted for WHO prequalification assessment shall be stated. If the test procedure in the IFU is changed in any way after completing verification and validation studies, the change(s) shall be reported to WHO, including a rationale for the change, and an explanation of why the study results support the claimed performance.

Specific information is provided in this document for the minimum numbers of lots of analysers and reagents/consumables (e.g. microcuvettes, control solutions, strips etc.) required for each study. Where more than one lot is required, each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents and components representative of routine manufacture. It is a manufacturer's responsibility to ensure, via risk analysis of its IVD that the minimum numbers of lots chosen for estimating performance characteristics considers the variability in performance likely to arise from the interlot diversity of critical components and their formulation or from changes that could occur during the commercial life of the IVD. Differences found between lots during the analytical and clinical performance studies shall be reported.

Estimation (and reporting) of IVD performance shall include the rate of invalid test results and the 2-sided 95% confidence interval around the estimated values for key performance metrics. The total percentage error shall be reported, and an explanation provided on how

[7] See WHO Prequalification document PQDx_173 for the list of recognized stringent regulatory authorities available on our website Prequalification Guidance | WHO - Prequalification of Medical Products (IVDs, Medicines, Vaccines and Immunization Devices, Vector Control)

it was calculated. The cause of invalid results/errors should be reported if available. Data shall be presented in a clear and understandable format. Discrepant results should be resolved as much as possible, however performance characteristics shall be based on the original result.

For analytical performance studies described in part 1 below it may be also possible to carefully design protocols that will generate useful data for more than one of the required studies, provided the specific criteria for each requirement are met by the study (e.g., number of replicates, concentration of analyte, etc.). Studies which may fall in this category are indicated in the appropriate chapters in the tables. In some analytical performance studies (where indicated) it is acceptable to use one specimen type, providing that the relationship between specimen types has been demonstrated by the manufacturer.

D. Table of Requirements

WHO requires that a product dossier is 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)(7). 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., 3.05.06, 3.05.08, etc). This has been done so as 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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IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
3.05.01 Stability of specimen(s)			
Specimen collection, storage and transport	Real time studies shall be determined taking into account: 1. Storage conditions (e.g. duration at different temperatures and variation in humidity, temperature limits, where appropriate). 2. Transport conditions, where applicable (see note 1). 3. Intended use. 4. Specimen collection and/or transfer devices recommended in the IFU, whether these contain anticoagulants and whether they can be sealed. 5. Testing shall be conducted in 1 lot. 6. The specimen panel shall contain a minimum of 15 specimens across the range of reference values in Table 1 in section C.1.	1. Evidence shall be provided which verifies the maximum allowable time between specimen collection, and its processing or addition to the IVD or storage in the setting where testing takes place. 2. Acceptance criteria will confirm that claimed specimen types transported, processed and stored under recommended conditions provided in the IFU will give expected results. Unless all specimens are expected to be processed as fresh samples within a specified time frame, the IVD performance shall be established for each different storage condition at the beginning and end of the stated period.	
3.05.02 Validation of specimens			
Demonstration of validity of all claimed specimen types	1. The relationship between IVD performance in all claimed specimen types (capillary blood, venous blood etc.) shall be established. 2. The specimen panel shall contain a minimum of 40 paired specimens (40 for each specimen type) across the range of reference values (Table 1). 3. Testing shall be conducted in 1 lot.	1. The entire process from the recommended specimen collection, processing and testing according to the IFU shall be followed. 2. The level of agreement for all specimen types, including each claimed anticoagulant shall be stated and the impact that this will have on each subsequent performance claim shall be fully understood and described. 3. Demonstration of equivalence between specimen types does not replace a clinical study (4.02.03).	WHO TGS-3 (*8*) CLSI EP35 (*9*)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
3.05.03 Metrological traceability of calibrators and control material values			
Metrological traceability of calibrators and control material values	**1.** As applicable, the metrological traceability of the provided control and calibration material(s) to a validated reference material (e.g. to Haemiglobincyanide WHO International Standard 98/708) or a secondary standard calibrated from it (e.g JCRMM912) shall be determined as well as identification of applicable reference materials and/or reference measurement procedures.	1. The version of the international standard used shall be stated. 2. Where the manufacturer controls are to be used with the IVD medical device then the value assignment process of the control material shall be described. 3. If third party control material is used for any part of the analytical or clinical performance analysis, then the manufacturer of the IVD shall provide relevant information from the manufacturer of the specified control materials where applicable.	CLSI H15-A3 (*10*)
3.05.04 Accuracy of measurement			
3.05.04.01a Trueness	Trueness of the IVD shall be estimated by comparison of the performance of the IVD with an established quantitative Hb reference method (see note 1). 1. Testing of 100 venous whole blood samples and 100 capillary blood samples with both the IVD and the reference method (see note 1). 2. The Hb concentration shall cover the entire linear range of the IVD. 3. A minimum of 2 lots of the reagents/strips/microcuvettes and 1 lot of instrument shall be used for the testing.	1. HiCn reference method shall be used. 2. Correlation of results between the IVD and the reference method shall be demonstrated statistically. 3. A difference plot (e.g. Bland-Altman) shall be provided presenting the results of the measurement procedure comparison, in order to visualize the underlying variability characteristics of this relationship. The horizontal axis of the plot should be the results from the reference method.	CLSI EP09 (*11*)
3.05.04.01b System accuracy	If a recognised comparator test is used instead of the reference method, comparative accuracy shall be demonstrated by comparison of the performance of the IVD	1. Refer to section “C.3” for criteria describing an established comparator test.	CLSI EP09 (*11*)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	under evaluation with an established Hb quantitative method (see note 1). 1. Testing of 100 venous whole blood samples and 100 capillary blood samples with both the IVD and the comparator method (see note 1). 2. The Hb concentration shall cover Hb concentration covering the entire linear range of the IVD. 3. A minimum of 2 lots of the reagents/strips/microcuvettes and 1 lot of instrument shall be used for the testing.	2. Correlation of results between the IVD and the established comparator method shall be demonstrated statistically. 3. A difference plot (e.g. Bland-Altman) shall be provided presenting the results of the measurement procedure comparison, in order to visualize the underlying variability characteristics of this relationship. The horizontal axis of the plot should be the mean of the 2 measurement procedure results.	
3.05.04.02 Precision (repeatability & reproducibility)	1. Both repeatability and reproducibility (see note 1) should be estimated using panels with defined analyte levels. 2. Repeatability and reproducibility specimen panels shall at least include (see note 2): • 1 non/mild-anaemic specimen; • 1 moderate anaemic specimens; • 1 severe anaemic specimen; • Control material if provided with the IVD. 3. Venous and capillary blood should be tested. 4. Each panel member shall be tested: • In 5 replicates; • Using 3 different lots of reagents/strips/microcuvettes and 3 different lots of analysers (note 3) and using the accessories recommended in the IFU or provided in the kit; • Over 5 days (not necessarily consecutive) with one run/day (alternating morning/afternoon); • At each of 3 different testing sites.	1. E.g. within- or between-run, -lot, -day, -site, -operator etc. 2. The concentrations of Hb in the specimens should span the linear range of the assay (see table 1). 3. Lots shall be composed of different batches of critical components. 4. Results must be statistically analyzed (e.g. using ANOVA to identify and isolate the sources and extent of any variance) . 5. The numbers of invalid tests must be reported. 6. To understand irregularities in results obtained, at least 2 of the 3 lots should be tested at each of the 3 testing sites. 7. 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 Usability/human factors – Flex studies). The results of estimating operator-to-operator variation on IVD	WHO TGS-3 (*8*) CLSI EP15-A3 (*12*) CLSI EP05-A3 (*13*)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	5. The effect of operator-to-operator variation on IVD performance is to be included as part of the precision studies. Testing shall be performed: • By 1 operator/site; • By operators representative of intended end users (see note 8); • Unassisted; • Using only the material provided with the IVD (e.g. IFU, labels and other instructional materials) and following the IFU procedure.	performance may be used in conjunction with studies to qualify the usability of the IVD. 8. Users should be selected based on a pre-determined and contextually appropriate level of education, literacy and auxiliary skills that will challenge the usability of the IVD and reflect the diversity of intended users and operational settings. These characteristics should be detailed in the submission.	
3.05.05 Analytical sensitivity			
Limit of blank	1. The limit of blank shall be determined by testing plasma specimens: • Obtained from 4 individuals; • Using 2 reagent lots; • At least 3 replicates per lot.	1. The plasma specimens should be confirmed to be haemoglobin free by the reference method.	
3.05.06 Analytical specificity			
Potentially interfering substances and medical conditions	1. The potential for false results (under or over quantification) arising from interference by the substances/conditions listed below shall be investigated. 2. Testing should be performed in non/mild-anaemic and severe anaemic specimens, in the presence or absence of each condition or potentially interfering substance at physiologically relevant dosages: • With each substance/condition represented by at least 3-5 specimens from different individuals (see note 5);	1. The risk assessment conducted for the IVD should identify substances at medically relevant level s that may interfere with the detection and appropriate interpretation of HbA1c, taking the device technology, specimen type and patient population into account. 2. By conducting appropriate risk assessment, testing can be conducted on specimens spiked with the substances identified as likely to be significant and	CLSI EP07 (*14*) CLSI EP37 (*15*) ISO 14971:2019 (*16*)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	• In 5 replicates; • In 1 claimed specimen type (venous whole blood); • In 1 lot.	testing of potentially irrelevant substances/conditions avoided. • Under some circumstances stringent risk evaluation may eliminate the requirement to test some of the items in the lists but any such decision shall be documented in any submissions to WHO and considered in the risk-benefit statements. 3. Any observed interference should be investigated and performance limitations of the IVD reported in the IFU. 4. 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. 5. Exogenous substances shall be spiked at the highest clinically relevant level compared with healthy individuals.	
Endogenous substances	1. The interference of endogenous substances listed below in whole blood on the performance of the device shall be investigated: • Triglycerides, lipoproteins, cholesterol, unconjugated bilirubin, albumin, creatinine, urea, uric acid, total protein; • Lipaemic specimens; • Leukocytes, platelets; • Abnormally high (54-65%) and abnormally low (17-18%) haematocrit. 2. The interference of following substances/conditions on the performance of the device shall be considered as per manufacturer's risk assessment: • Hb deviations such as elevated carboxyhemoglobin levels, methemoglobin levels; • Sickle cell anaemia, thalassemias, variant haemoglobin (A, D, E, S, C) anaemias, polycythemia vera, haemoglobinopathies, iron deficiency, leukaemia, and/or other red blood cell dyscrasias.		
Exogenous substances	The interference of exogenous substances on the performance of the device shall be investigated as per manufacturer's risk assessment. The interference of exogenous substances on the performance of the device shall be investigated, such as:		

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	1. Representatives from each of the following classes of drugs: antibiotic, anti-tuberculosis, antiretroviral drugs, cancer chemotherapies, anticoagulants, blood pressure and cholesterol lowering drugs. 2. Common over-the-counter analgesic medications (such as aspirin, paracetamol, ibuprofen). 3. Ethanol, caffeine. 4. Aldosterone-related steroid. 5. Intravascular dyes, such as indocyanine green or methylene blue.		
3.05.08 Measuring range of the assay			
3.05.08a Linearity	The linear range shall be established using: 1. A dilution series with 7-11 concentrations that span and exceed the expected upper and lower limits of the measuring range shall be tested. 2. 2 to 4 replicates shall be tested at each concentration. 3. Using 1 reagent lot. 4. Using 1 specimen type (e.g. venous blood).	1. Quantification of the parent material used to make the dilution series with at least two suitable Hb quantitative assays (see section C.3). 2. The lower part of the measuring range shall be determined using the Hb IS or a secondary standard calibrated against it. 3. The upper part of the measuring range may be established using dilution series of a clinical specimen with a high Hb concentration. 4. The test results shall be analysed using appropriate statistical tools (e.g. Deming Regression Analysis) to demonstrate correlation between the IVD results and the nominal concentrations of the analyte.	CLSI EP06 (*17*)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
3.05.08b Limits of quantitation	Lower and upper limits of quantitation (LLOQ, ULOQ)[8] of Hb shall be established. 1. For the LLOQ determination, a minimum of 15 (3 days, 5 replicates/day) replicate tests of a multi-member dilution panel of a suitable biological reference material (e.g. Haemiglobincyanide WHO International Standard 98/708) or a secondary standard calibrated against it) shall be tested (see note 3). 2. For the ULOQ determination, a dilution series prepared from a highly concentrated clinical specimen shall be tested (see note 1). 3. The concentrations of the dilution panel shall go beyond the claimed LLOQ and ULOQ. 4. Testing shall be undertaken using 2 reagent lots. 5. LLOQ and ULOQ shall be estimated by determining the lower and upper concentrations that can be determined within the accuracy expected (predefined) (see note 2). 6. All claimed specimen types shall be tested.	1. In order to determine the ULOQ accurately, it may be necessary to use a parent specimen with high Hb concentration calibrated against the IS to spike specimens to obtain large volumes of highly concentrated material. 2. Predefined criteria for acceptable accuracy (precision & trueness) at the LLOQ and the ULOQ shall be provided. 3. The version of the IS used shall be stated (where applicable).	CLSI EP17-A2 (*18*) PQDx_018 (*2*) CLSI EP05-A3 (*13*)
3.05.10 Validation of the assay procedures			
Validation of assay parameters	1. Evidence shall be provided demonstrating how parameters (specified in the IFU) were determined, verified and validated (see note 2).	1. The parameters may be investigated as part of 3.06.04 Usability/human factors studies. Provide a cross reference if the studies are submitted in other sections of the dossier.	IMDRF TOC (*7*) PQDx_18 (*2*)

[8] Limit of quantitation (LoQ): the lower and upper concentrations at which precision & trueness are within specified criteria

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	2. Testing using 1 non/mild-anaemic specimen and 1 severe anaemic specimen. 3. In 1 specimen type only (venous blood). 4. Validation shall be performed at each concentration using a minimum of 2 different reagent lots: • 1 freshly made lot; • 1 lot towards the end of its assigned shelf life. 5. The following parameters shall be considered depending on the assay and IFU requirements (see note 1, 2, 3): • Time between drawing specimen, handling and loading; • Operating temperature, humidity (see note 4 and 5); • Varying specimen volume spanning the limits of the IVD (within tolerance levels): reduced blood volume to excess volume of specimen; • Error codes for specimens outside the measuring range.	2. The extent of the assay parameter validation shall be subject to a documented risk assessment. 3. The intent of assay parameter validation is to demonstrate that a combination of small but defined deviations of the parameters outlined in the IFU will not result in inaccurate results i.e., to demonstrate the assay is robust. 4. Performance studies shall be conducted at the extremes of the intended operational temperature range. The number of invalid results shall be recorded for each temperature investigated. 5. The ranges of humidity tested shall be risk-based, taking into consideration likely operational settings in resource limited settings.	
3.06.02.08 Software verification and validation			
3.06.02.08a Software validation	1. Software validation reports shall be available for submission if requested (see note 1).	1. Software validation to include as a minimum: • Verification of built-in fail-safe; • Verification of alert mechanisms; • Verification of quantitative results detection; • Verification of quantitative results calculation.	IEC 62304:2006/ Amd 1:2015 (*19*) U.S FDA (*20*, *21*)
3.06.02.08b Error codes	1. Manufacturer shall provide a list of all error codes the instrument can display to the end user .	1. Evidence to demonstrate that appropriate error codes are provided to the end user shall be available for submission if requested.	

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
3.06.03 Cleaning and disinfection validation			
Cleaning and disinfection validation	1. Disinfection efficacy studies shall be performed to demonstrate: • Efficacy of the chosen disinfectant against blood-borne pathogens (see note 2). At a minimum efficacy shall be demonstrated against Hepatitis B virus as it is the most difficult to kill; • Efficacy of the cleaning and disinfection procedure with the analyser external components e.g., case, display, buttons, etc.; • That the analytical performance of the analyser is not impacted (even after multiple cleaning and disinfection cycles); • That the functionality of the analyser components and features, including reagent system port and any parts particularly susceptible to blood contamination, are not impacted (even after multiple cleaning and disinfection cycles). 2. Physical indicators of deterioration (to the screen, buttons, plastic housing) during the cleaning and disinfection phase shall be evaluated and this information shall be included in the study. 3. Demonstrate that accuracy is not affected by repeated cleaning and disinfection.	1. The studies conducted shall be based on the design of the device and risk assessment. • Infection control considerations and measures shall be documented in the risk analysis and risk assessment. 2. At the very least, the disinfectant product shall be effective against HIV, Hepatitis C, and Hepatitis B viruses.	ASTM E1053-20 (*22*) US FDA (*23*)
3.06.04 Usability/human factors			
3.06.04a Flex studies/ robustness	1. The influence of the following factors on expected results (non/mild-anaemia and severe anaemia) should be considered, if appropriate:	1. The risk assessment conducted for an IVD shall identify factors which have potential to affect the performance of the assay	WHO PQDx_018 (*2*) U.S FDA (*24*)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	• Temperature (see note 4); • Time between drawing blood specimen, handling and loading; • Specimen and/or reagent volume; • Lighting, humidity and barometric pressure (simulating high altitude); • Dust; • IVD instrument sturdiness (including the effect of non-level work surface); • Handling contamination (e.g. from alcohol, hand sanitizer, latex, powder, hand lotion, sweat, and/or soap); • Anticoagulants (e.g. K_2EDTA, Li-Heparin). 2. Testing to be performed in 1 lot.	2. Refer to WHO document PQDx_018 "Instructions for compilation of a product dossier" (*2*) for other flex studies that may be relevant, taking into consideration the broad range of operational and environmental conditions consistent with intended use. 3. Studies investigating the impact of specimen volume shall be conducted in all specimen types. 4. The factors 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 can be understood. For example, in addition to investigating deviations of temperature within those claimed in the IFU (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). 5. For the purposes of this document, ruggedness means the ability to resist environmental shocks of a variety of kinds. 6. Robustness testing generally takes the form of statistically designed experiments to evaluate the effect of simultaneous "small but deliberate changes" in method parameters.	
3.06.04b Qualification of usability: Label	1. Questionnaire-based testing of subjects to assess ability of intended users to correctly comprehend key messages from packaging and labelling:	1. Instructions for use and labelling should be clear and easy to understand; use of pictorial instructional material is encouraged.	IEC 62366-1:2015 (*25*) U.S FDA (*24*)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
comprehension study	• Understanding key warnings, limitations and/or restrictions; • Test procedure comprehension; • Ease of following instructions. 2. Questionnaire to be administered to at least 15 intended users, including those whose native language may not be the language of the IFU if necessary, to demonstrate comprehension of key messages.	2. Prequalified Hb POC IVD users will include trained lay providers and trained health care workers. For prequalification purposes, these should be considered as the intended user, rather than only laboratory professionals. Manufacturer staff should be excluded.	
3.06.04c Qualification of usability: Results interpretation study	1. Intended users shall be requested to interpret key symbols provided to guide interpretation of the outputs (including errors) of the Hb analyser (see note 1, 2). 2. Testing subjects to consist of at least 15 intended users to demonstrate correct interpretation of test results.	1. Study group may include subjects recruited as part of the label comprehension study. 2. The manufacturer shall include a range of Hb concentrations that trigger different status/key symbols (including range of error messages) on the device. This can be partially conducted using fresh whole blood specimens taken from a range of pre-screened Hb specimens.	
3.06.05 Stability of the IVD			
3.06.05.01 & 3.06.05.03 Claimed shelf life including transport stability	1. Stability studies shall be conducted using the conditions expected in the environment of intended use. 2. Lots shall be subjected to simulated "transport stress" before real time studies are undertaken on these lots. 3. Lots shall be subject to simulated environmental stress conditions (e.g. temperature and humidity). 4. The effects of this simulated transport shall be documented separately and in addition to the real time studies. 5. Real time shelf-life studies shall evaluate the storage temperature and humidity range.	1. Acceptance criteria shall be defined in advance. 2. Lots shall comprise different batches of critical components. 3. Determination of shipping stability shall be performed using simulated extreme stress conditions, ensuring that application of those conditions is consistent and controlled. 4. 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	ISO 23640:2011 (*26*) CLSI EP25 (*27*) WHO TGS-2 (*28*) WHO Annex TGS-2 (*29*) ASTM D4169-22 (*30*)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	6. At least 3 lots shall be tested. 7. The stability panel shall consist of the following specimens: • 25 non-anaemic specimens; • 25 mild anaemic specimens; • 25 moderate anaemic specimens; • 25 severe anaemic specimens. 8. Each panel member shall be tested in triplicate at each time point/condition. 9. Venous blood shall be tested. 10. Multiple instruments may be used to allow simultaneous testing at each time point. 11. Lots shall be subject to simulated physical stress conditions (e.g. drop-shock, inversion, vibration, physical handling and stacking).	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. 5. Accelerated studies do not replace the need for real time studies. 6. In-use stability of labile components shall be conducted using components in their final configuration. 7. The number of invalid tests with each kit lot shall be reported. 8. The effects of light on labelling and to the kit contents shall be investigated if identified in the risk analysis.	
3.06.05.02 In-use stability (open pack or open vial stability)	1. In-use stability testing shall be performed on a minimum of 1 lot. 2. Testing in triplicate shall be undertaken using a stability panel composed of: • 1 severe anaemic specimen; • 1 non/mild-anaemic specimen. 3. All labile components shall be evaluated (e.g. buffers vials, sealed cartridges, etc., see note 6). 4. Only 1 claimed specimen type is required to be tested.		

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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			
Clinical evaluation study	1. Testing shall be conducted: • On specimens from all sections of the population (across the stated age range, including pregnant women, children, malaria patients); • In different geographical settings; • representative of intended use (minimum of 2 regions); • By a variety of intended users representing relevant intended use settings (e.g., different levels of health care facilities) (see note 1); • Using at least 2 lots of both the analyser and reagents/consumables (see note 3). 2. All specimens shall be tested by the comparator test (see note 5). 3. Specimens with discrepant results shall be further evaluated. Where possible, follow-up testing shall be done to determine the cause (see note 10). 4. The procedure for selection of study subjects, how these represent the intended use population and how bias has been addressed shall be clearly described.	1. Prequalified Hb POC IVDs will generally be used by trained lay providers and trained health care workers in point of care settings. For prequalification purposes, these shall be considered as the intended user/setting, rather than a laboratory professional. • In addition, the operator shall not be linked in any way to the manufacture of the device. 2. Problematic specimens, those with unexpected results but which otherwise meet selection criteria for a study, shall not be systematically excluded from analysis. 3. Each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents, representative of routine manufacture. 4. A separate, venous whole blood specimen shall be collected in parallel to establish the comparator result. 5. The comparator test used shall meet the characteristics outlined in section C.3. • It shall give a quantitative determination of Hb concentration, expressed as g/dL, g/L or mmol/L; • Additionally the comparator test shall be well maintained and verified with quality control material at the site of the study.	CLSI H26-A2 (*31*)

IMDRF ToC Chapter heading and aspect	Testing requirements	Notes on testing requirement	Source documents
	5. Testing shall be conducted in individual subjects. Testing shall be conducted for both capillary and venous blood using a minimum of: • 200 subject specimens with mild anaemia; • 200 subject specimens with moderate anaemia; • 200 subject specimens with severe anaemia; • 200 non-anaemic subject specimens, including specimens within the 131-170 g/L and > 171 g/L Hb.	6. All results shall be included in the denominator data for analysis. 7. All invalid results shall be recorded and evaluated in comparison to the comparator result. Invalid results should be analyzed separately in the final performance calculations. 8. Correlation between the IVD and the comparator method shall be established statistically. 9. Clinical performance study protocols shall specify how results from the IVD under evaluation and the comparator assay will be compared and how results in the two assays will be statistically determined to be equivalent or not (e.g. Bland Altman analysis). 10. Problematic specimens, and those specimens with initial discrepant results shall not be excluded from the final analysis.	

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E. Source documents

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