Technical specifications series for submission to WHO prequalification – diagnostic assessment

TSS-15

In vitro diagnostic medical devices used for the quantitative detection of Hepatitis B DNA
In vitro diagnostic medical devices used for the quantitative detection of Hepatitis B DNA
(Technical specifications series for submission to WHO prequalification – diagnostic assessment, TSS15)

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1 Joined by teleconference
2 Joined by teleconference
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ALT</td>
<td>alanine transaminase</td>
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<tr>
<td>anti-HBe</td>
<td>antibody to hepatitis Be antigen</td>
</tr>
<tr>
<td>anti-HBsAg</td>
<td>antibody to hepatitis B surface antigen</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>HBV</td>
<td>hepatitis B virus</td>
</tr>
<tr>
<td>HBeAg</td>
<td>hepatitis B early antigen</td>
</tr>
<tr>
<td>HBsAg</td>
<td>hepatitis B surface antigen</td>
</tr>
<tr>
<td>IFU</td>
<td>instructions for use</td>
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<tr>
<td>IS</td>
<td>International Standard</td>
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<tr>
<td>IVD</td>
<td>in vitro diagnostic</td>
</tr>
<tr>
<td>IU</td>
<td>international units</td>
</tr>
<tr>
<td>LOD</td>
<td>limit of detection</td>
</tr>
<tr>
<td>LLOQ/ULOQ</td>
<td>lower limit of quantitation / upper limit of quantitation</td>
</tr>
<tr>
<td>NAT</td>
<td>nucleic acid amplification technology</td>
</tr>
<tr>
<td>NPV</td>
<td>negative predictive value</td>
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<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>POC</td>
<td>point of care</td>
</tr>
<tr>
<td>PPV</td>
<td>positive predictive value</td>
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<td>WHO</td>
<td>World Health Organization</td>
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1. 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 of tests for the quantification of hepatitis B virus (HBV) DNA using nucleic acid amplification techniques. Blood screening assays are beyond the scope of this document.

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 requirements.

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.

Where possible, WHO analytical and clinical performance study requirements 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.

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.

2. How to apply these specifications

For purposes of WHO prequalification, HBV DNA detection assays shall comply with the specifications in Part 1 and Part 2 of this document. Part 3 only applies if, according to the IFU, testing is performed by healthcare professionals in near point of care (POC) settings.

3. Other guidance documents

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

WHO prequalification documents
- Technical guidance series for WHO prequalification - diagnostic assessment

3 Available at https://extranet.who.int/pqweb/vitro-diagnostics/guidance-documents
Instructions for compilation of a product dossier, WHO document PQDx_018

WHO Global Hepatitis programme guidelines:
- Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection (1)
- Guidelines on hepatitis B and C testing (2)
- Guidelines on hepatitis B and C testing - Policy brief (3)

4. Performance principles for WHO prequalification

4.1 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 and what is measured (e.g., HBV DNA from genotypes A to H)
- The clinical indication and function of the IVD (e.g., quantitation of HBV DNA for monitoring of HBV anti-viral therapy, monitoring of disease progression);
- What the IVD reports/the result output;
- The target population (e.g., see WHO Global Hepatitis programme guidelines);
- Whether or not it includes automated components or is intended to be used with automated instruments;
- The intended use environment (e.g., laboratory setting, and/or near-POC4);
- The intended user;
- The intended specimen type; and
- Any limitations to the intended use e.g., not intended for blood screening, identification or restrictions regarding age groups or other limiting characteristics.

4.2 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., serum, plasma, venous whole blood, capillary whole blood, dried blood spot (DBS)) claimed in the IFU. For DBS specimens, the validated brand(s) of filter paper shall be stated in the IFU.

Prequalified HBV DNA NAT assays in low- and middle-income countries are likely to be used by a range of users in different geographical settings:
- laboratory professionals5 either in centralised testing laboratories or at near-POC,
- laboratory professionals in health care settings not experienced in nucleic acid testing,
- health professionals trained in the use of the test at near-POC.

Depending on the intended use of the IVD, analytical and clinical performance studies shall be designed to consider not only the diversity of knowledge and skills across the population of individuals using the IVD, but also the likely operational settings in which testing will occur, and the genetic variability of hepatitis B in the intended test populations.

4 In some jurisdictions (e.g., European Union), the concept “near patient testing” is used instead of “point of care testing”. Either term may be used in the intended use statement.
5 Medical technologists, medical laboratory technicians or similar, who have received a formal professional or paraprofessional certification or tertiary education degree.
It is a manufacturer’s responsibility to ensure that the risk assessment for an IVD reflects the intended operational settings, including laboratory or service delivery complexity, user expertise, training received and test population.

4.3 Applicability of supporting evidence to IVD under review

Analytical and clinical performance studies shall be undertaken using the specific, final (locked-down) version of the assay 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 (4). If the protocol section of the IFU has been changed in any way, both the protocol provided to laboratory for clinical performance studies as outlined in Part 2 of this document and that in the final version of the IFU intended for users shall be provided with the submission to WHO prequalification.

The version of the IFU used for performance evaluations submitted to WHO prequalification shall be stated. If the test procedure in the IFU is changed in any way after completing performance verification and validation studies the change 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 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, 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 assigned shelf life of the IVD where applicable. Differences found between lots during the analytical and clinical performance studies shall be reported. Where the manufacturer supplies additional instrumentation required to conduct the assay, safety and performance data shall be provided in the dossier with this instrumentation.

The true HBV DNA status/concentration of a specimen used in analytical or clinical performance studies shall be determined using a suitable reference algorithm/state of the art test, for which justification shall be provided (such as a laboratory based assay that has undergone comprehensive pre-market assessment and has been stringently regulated and approved by a recognized regulatory authority6). For WHO purposes this should be to a method that is currently at a developed stage of technical capability based on the relevant consolidated findings of science, technology and experience (commonly referred to as state of the art).

Discrepant results should be resolved as much as possible, however performance characteristics shall be based on the original result. Comparison with a similar device detecting the same genomic target is insufficient for resolution of discrepant results.

Estimation (and reporting) of IVD performance shall include the rate of invalid test results and the two-sided 95% confidence interval around the estimated values for key performance metrics. The cause of invalid results should be reported if available (such as

6 See document PQDx_173 Abridged Assessment for the list of recognized stringent assessments by regulatory authorities.
sample issues (e.g., age of sample, storage conditions, inadequate sample volume), instrument hardware and/or software error, operator error. Data should be presented in a clear and understandable format.

For all analytical performance studies listed in part 1, it is unlikely that clinical specimens will be available in the volumes required. Therefore, it is acceptable to use contrived specimens, for example, a well characterised clinical specimen spiked into the appropriate matrix, i.e., a matrix that has been claimed in the intended use of the IVD (e.g., human plasma, whole blood), and which has been prepared in a validated and standardized manner for such studies. In addition, dilutions of a high-concentration clinical specimen may be used, if they are in an appropriate matrix (e.g., plasma, whole blood) for certain studies, e.g., limit of detection (LOD) studies. The material chosen should use the entire assay system from specimen preparation to interpretation.

For certain analytical performance studies (part 1) it may be acceptable to generate evidence of performance using a single genotype as a surrogate for performance of other genotypes claimed in the intended use of the device.

For part 1 it may be also possible to carefully design a study which 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, sample types, etc.). For example, precision testing and whole system failure testing could be combined in a single study. Studies which may fall in this category are indicated in the appropriate sections of part 1.

Clinical performance studies shall be based on testing human specimens only sourced from population cohorts reflective of the intended use. Independent of the outcome of the equivalency study (section 1.2.1), all claimed specimen types need to be considered in the clinical performance study, unless otherwise stated in part 2. The use of well-characterised repository specimens and panels may be acceptable if they are relevant to the IVD under assessment, taking into consideration:

- storage conditions (e.g., including age of the specimen, temperature logs, freeze-thaw cycles if applicable);
- the stability of the nucleic acid target;
- selection bias.

Studies that comprise the testing of left-over specimens by research and development staff at a manufacturer’s facility shall not, on their own, be considered sufficient to meet the clinical performance study requirements summarized in this document.
### PART 1  
**ANALYTICAL PERFORMANCE AND OTHER EVIDENCE**

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### PART 3  
**QUALIFICATION OF USABILITY FOR NEAR POINT OF CARE TESTING BY HEALTHCARE PROFESSIONALS**

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## Part 1: Analytical performance and other evidence

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<th>Notes on testing requirements</th>
<th>Source documents</th>
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<tr>
<td>1.1 Stability of specimen(s)</td>
<td>1. Real time studies shall be conducted for each specimen type (e.g., plasma, serum, whole blood, dried blood spot) using the specimen collection and/or transfer devices intended to be used with the IVD where relevant and taking into account: • required transport conditions • storage conditions (e.g., duration at different temperatures, temperature limits, freeze/thaw cycles etc.). For DBS specimens, humidity shall be evaluated • intended use (see note 1) 2. A minimum of 10 weak positive specimens (approx. 3 x LLOQ) shall be used (see note 2) 3. Testing shall be conducted in 1 lot</td>
<td>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. Specimens of the desired reactivity may be manufactured by diluting a natural positive specimen with negative clinical matrix 3. In case the use of archived specimens is considered for Part 2 of this document, evidence of stability in the conditions in which the specimens have been stored shall be demonstrated e.g., by re-testing a subset of specimens with an approved test to verify that the same reactivity/result is obtained compared to the sample result prior to freezing 4. Acceptance criteria will confirm that claimed specimen types transported, processed and stored under recommended conditions will give expected results. Separated EDTA plasma and centrifuged whole blood in a plasma preparation tube are considered different specimen types in this context 5. The manufacturer shall define what the acceptable deviation is when reporting their results in the study report 6. If DBS specimens are a claimed specimen type, the details of the filter paper (brand, product code) shall be specified, and the use and stability validated 7. 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</td>
<td>TGS 3 (S)</td>
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### 1.2 Validation of specimens

#### 1.2.1 Demonstration of equivalence between specimen types

The relationship between IVD performance in claimed specimen types shall be established:

1. At least 25 positive and 25 negative specimens shall be tested for each claimed specimen type (see note 1, 2).
2. If testing is claimed in specimens other than blood derived specimens, then testing shall be conducted in at least 50 paired specimens (positive and negative).
3. For assays that detect more than one genotype but do not differentiate the genotype, the justification as to whether to study each genotype shall be risk- and evidence-based.
4. 1 replicate of each specimen for each specimen type shall be tested.
5. At least 1 lot shall be used for testing.

- If multiple specimen types are claimed, (e.g., serum, plasma, venous whole blood, capillary whole blood, DBS from capillary whole blood), then equivalence shall be demonstrated using paired specimens; in other words, demonstrated in each specimen type for all specimens.
- Similarly, if equivalence is claimed in specimens collected into multiple anticoagulants, this shall be demonstrated for each of the claimed anticoagulants.

2. Specimens should be chosen that have low (x 3 LLOQ) to medium concentrations of the analyte. It can be expected that IVDs may show different sensitivities between specimen types. The manufacturer shall define what the acceptable deviation is in the study report.

3. The established relationship between IVD performance in claimed specimen types (plasma, blood, DBS) shall be considered in the design of subsequent studies. For example, if the studies show that one or more of the claimed specimen types are equivalent, then not all specimen type need to be tested in some of the subsequent studies (where indicated).

### 1.3 Metrological traceability of calibrator and control material values

#### 1.3.1 Metrological traceability of calibrator and control material values

1. As applicable, the metrological traceability of control and calibration material(s) to a validated reference material or a secondary standard calibrated from it shall be determined (e.g., to WHO International Standard (IS) Hepatitis B Virus DNA).
2. Material with well characterized viral load should only be used in cases where material with an assigned value in International Units (IU) is not available. However, this viral load shall have been determined with a method considered stringently assessed (see chapter 4.3).

- The version of the IS used shall be stated.
- Appropriate to the IFU, the positive controls contain a defined amount of target in a suitable matrix. The low positive external control should contain target nucleic acid at levels approximately 3 x LLOQ. The high positive external control should contain target nucleic acid at levels in the upper half of the linear range of the assay.

3. An internal control shall be added to each specimen before sample extraction so that all stages of the test, from extraction to final target detection, can be verified. An internal control consists of a defined nontarget sequence of the same type of nucleic acid as the target, which are extracted and amplified simultaneously with the test sample). Therefore, the test should be able to clearly identify and...
distinguish the amplified products (amplicons) of the internal control and the target
4. Some quantitative assays may include an internal calibrator which is assayed simultaneously with the target to allow for quantitation of specimens based on algorithms that compare signal generated from the internal calibrator and target sequence to a pre-generated standard curve. In such cases, the establishment of the stored/pre-generated standard curve shall be validated. If calibration constants are used, the interval between recalibration of the pre-generated standard curves and calibration constants shall also be validated

1.4 Accuracy of measurement

| 1.4.1 Trueness | 1. The trueness of the IVD shall be demonstrated by comparison of the performance of the IVD with an established reference method (see note 1)  
2. The following specimens shall be tested by the IVD and the reference method:  
   • A total of at least 100 specimens positive for HBV DNA with viral loads covering the entire linear range of the IVD where relevant and including as many different genotypes/subtypes as possible  
3. A minimum of 2 lots shall be used for the testing | 1. The reference method shall be stringently regulated and approved by a recognized stringent regulatory authority\(^7\) and acknowledged in the literature as representing state of the art  
2. Testing is only required in each specimen type if the specimen types are not equivalent (see section 1.2)  
3. Either contrived specimens with varying viral loads or clinical specimens may be used  
4. Correlation of results between the IVD and the reference method shall be demonstrated statistically  
5. Trueness may be established during the clinical performance studies in part 2 of this document | CLSI EP09-A3 (11)  
CLSI EP15-A3 (12) |
|---|---|---|
| 1.4.2 Precision (Repeatability & reproducibility) | 1. Both repeatability (see note 1) and reproducibility (see note 2) shall be estimated using panels with defined analyte levels (see note 4)  
2. The members of the repeatability and reproducibility panel shall include (see note 3):  
   • 1 x negative specimen  
   • 1 x low positive specimens (approx. 3 x LLOQ)  
   • 2 x specimens in the middle and upper range of the linearity span, respectively | 1. Within run  
2. Between -run, -lot, -site, -operator  
   • A run will be defined depending on the IVD’s throughput: if the platform can accommodate all specimens in a single run, i.e., in the same test plate, the specimens will be run together. If the assay can only accommodate a smaller set or a single specimen(s), a run will be defined as a testing session carried out on the same instrument/module on the same day  
3. Specimens with target levels of analyte may be contrived | TGS 3 (5)  
CLSI EP05-A3 (13)  
CLSI EP12-A2 (14) |

\(^7\) See document PQDx_173 Abridged Assessment for the list of recognized stringent assessments by regulatory authorities
- Positive specimens in the panel shall all be of the same genotype
- Each panel member shall be tested (see note 10):
  - in 5 replicates
  - using 3 different lots (see notes 6 & 9)
  - over 5 days (not necessarily consecutive) with one run in that day (if possible alternating morning/afternoon) or >1 run in that day
  - at each of 3 different testing sites (see note 5)
4. Testing shall be conducted by:
   - by 1 operator/site (see note 4, 5)
   - by operators representative of intended users in addition to members of manufacturer’s staff
   - unassisted
   - using only those materials provided with the IVD (e.g., IFU, labels and other instructional material)
5. All claimed specimen types shall be tested. If equivalence between claimed anticoagulants has been demonstrated, only 1 anticoagulant is required to be tested.

### 1.5 Analytical sensitivity

#### 1.5.1 Limit of detection

Analytical sensitivity shall be estimated as the concentration of HBV DNA detectable 95% of the time, otherwise known as limit of detection (LOD).

1. The determination shall comprise 20 - 24 replicate tests (8 replicate tests on each of 3 days) of a minimum 8-member 0.5log_{10} dilution panel of a suitable biological reference material (e.g., WHO International Standard for HBV DNA for NAT or a secondary standard calibrated against it)
2. The replicate testing shall be conducted on 3 different days (see note 3)
3. The panel members’ HBV DNA levels shall span DNA concentrations below and above the IVD’s LOD
4. At least 2 dilution series shall be tested
5. LOD shall be estimated for all specimen types (see note 5)

4. The testing panel should be the same for all operators, lots and sites.
5. If operators are considered a significant source of test result variation (for example, with tests that have a significant proportion of manual manipulations), then at least 2 operators/site shall be used
6. Each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents, representative of routine manufacture
7. The number of invalid tests shall be reported
8. Results shall be statistically analysed (e.g., ANOVA or other method) to identify and isolate the sources and extent of any variance. In addition, 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.
9. To understand irregularities in results obtained, at least 2 lots should be tested at each of the 3 testing sites
10. Alternative methods used to establish repeatability and reproducibility performance of the assay shall be discussed with WHO in advance of dossier submission

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| 1. | The version of the international standard (IS) used shall be stated.
| 2. | LOD for HBV DNA shall be expressed in international units with a 95% confidence interval that takes into account lot to lot variation
| 3. | For low throughput instruments, the number of testing days may be increased
| 4. | The test results will be analysed using appropriate statistical tools (e.g., probit analysis)
| 5. | The relationship between analytical sensitivity for the different claimed specimen types (e.g., EDTA plasma and whole blood) shall be established. The design of subsequent analytical performance studies shall take that relationship into account to ensure that results of different specimen types, when included in the study, can be evaluated

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CLSI MM06-A2 (8)
CLSI EP12-A2 (14)
Commission implementing regulation (EU) 2022/1107 (15)
CLSI EP17-A2 (16)
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<tr>
<td>6.</td>
<td>Testing shall be conducted using a minimum of 2 different lots (see chapter 4.3) (see note 2)</td>
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<tr>
<td>7.</td>
<td>LOD shall be verified for HBV genotypes B - H using 1 lot (see note 6)</td>
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</table>

1.5.2 **Limits of quantitation**

Lower and upper limits of quantitation (LLOQ, ULOQ) (see note 1) shall be estimated by determining the lower and upper concentrations that can be determined within the accuracy expected (predefined):

1. The LLOQ determination shall comprise a minimum of 15 (3 days, 5 replicates per day) replicate tests of a multi-member dilution panel of a suitable biological reference material (e.g., WHO HBV DNA IS or a secondary standard calibrated against the appropriate IS)
   - For the ULOQ, determination, a dilution series prepared from a high-titre clinical specimen isolate shall be tested in a similar manner (see note 4)
2. The concentrations of the dilution panel members shall go beyond the claimed LLOQ and ULOQ
3. LLOQ shall be estimated for each claimed genotype
4. For other genotypes of HBV for which an IS is not available, the reference material shall be quantitated with a suitable HBV quantitative assay, approved by a recognized stringent regulatory authority
   - LLOQ will be determined using a minimum of 4 well characterised positive clinical specimens
5. LLOQ shall be estimated for each claimed specimen type
6. The replicate testing shall be conducted on 3 different days
7. Using 2 different lots (see chapter 4.3)

1. Limit of quantitation (LoQ): the lower and upper concentrations at which precision & trueness are within specified criteria
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
4. ULOQ should be estimated for each specimen type

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European Pharmacopoeia (9)
CLSI EP05-A3 (13)
Commission implementing regulation (EU) 2022/1107 (15)
CLSI EP17-A2 (16)
PQDx_018 (17)
### 1.6 Analytical specificity

#### 1.6.1 Potentially interfering substances and medical conditions

The potential for false results arising from interference from at least the substances/conditions (see note 1) listed below shall be determined by testing confirmed HBV negative samples, both spiked (using a single, common HBV genotype) and unspiked:

1. Substances/conditions represented, where possible, by at least 5–10 specimens from different individuals
2. A minimum of 100 specimens shall be tested
3. Spiking with HBV at approx. 3 x LLOQ
4. Using only 1 claimed specimen type

#### 1.6.1.1 Endogenous

The interference of endogenous substances in human plasma on the performance of the device shall be investigated, such as (see note 4):

1. Human genomic DNA
2. Haemoglobin, lipids, bilirubin, albumin
3. Elevated liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT)
4. Autoimmune diseases/markers:
   - Rheumatoid factor
   - Systemic lupus erythematosus
   - Anti-nuclear antibodies

#### 1.6.1.2 Exogenous

The interference of exogenous substances on the performance of the device shall be investigated (see note 3, 5), such as:

1. Medicines, relevant to the populations intended to be tested including: antiviral medications used in treatment of hepatitis B, antiretrovirals, interferon, other anti-parasitic, antimalarial and anti-tuberculosis drugs
2. Common over-the-counter anti-inflammatory medications (aspirin, paracetamol)
3. Medicines used to treat infections common in the region where the IVD will be used

---

1. The risk assessment conducted for an IVD shall identify substances where the potential for interference can reasonably be expected with the measurand to be detected (i.e., HBV DNA) in the areas of intended use and not simply rely on published lists of such compounds and conditions which might be of limited relevance in resource limited settings
   - By conducting appropriate risk assessment, testing can be conducted on specimens spiked with the substances/conditions identified as likely to be significant and 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 taken into account in the risk-benefit statements
2. Endogenous substances shall be spiked at abnormally high levels compared with healthy individuals
3. Exogenous substances shall be spiked at >3 times the peak plasma levels
4. Where clinical specimens from individuals with the disease state to be tested are unavailable, a negative specimen shall be spiked with the organism of interest to a high concentration (a minimum of 10^5 plaque forming units/mL for viruses and 10^6 colony forming units/mL for bacteria)
5. Any observed interference or cross-reactivity shall be investigated and performance limitations of the IVD reported in the IFU and taken into consideration in the required risk - benefit statements
6. Results shall be reported with respect to each condition and not be reported in relation to the total number of specimens tested in the study
7. Any observed cross-reactivity shall be further investigated and performance limitations of the IVD reported in the IFU

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Commission implementing regulation (EU) 2022/1107 (15)
CLSI EP07 (18)
CLSI EP37 (19)
| 1.6.2 Cross-reactivity | 1. The potential for false positive results shall be determined arising from cross-reactivity (see note 1) with other organisms unrelated to hepatitis B or disease states commonly found in regions of the intended use of the IVD, including where possible, at least 3–5 of the commonly found viruses (see note 4, 5, 6), such as:

**Viruses, such as:**
- HIV 1 & 2
- Hepatitis A, C, D, E viruses
- BK virus
- Cytomegalovirus,
- Epstein–Barr virus,
- Varicella zoster virus,
- Herpes simplex 1 & 2
- HTLV I &II
- Human herpes viruses
- Parvovirus B19

**Bacteria/parasites/fungi, such as:**
- *Plasmodium* spp
- *Leishmania*
- *Trypanosoma cruzi*
- *Trypanosoma brucei*
- *Staphylococcus aureus*
- *Staphylococcus epidermis*
- *Propionibacterium acnes*
- *Neisseria gonorrhoea*
- *Candida albicans*

2. Using 1 claimed specimen type
## 1.7 Measuring range of the assay

### 1.7.1 Linearity

The linear range shall be established using:

1. A dilution series with a minimum of 7 concentrations that span and is 20 to 30% wider than the expected upper and lower limits of the measuring range shall be tested.
2. At least 3 replicates tested at each concentration.
3. Lower part of the measuring range can be determined using the HBV IS or well characterised clinical material for genotypes other than genotype A (see note 3).
4. Using 1 lot.
5. All claimed specimen types shall be tested.
6. All major HBV genotypes that are predominant in populations for which claims are made and all other genotypes/subtypes, if available, that are claimed in the IFU shall be included in the dilution series to establish the linearity of the assay across all tested genotypes.

Data for establishing the linear range can be taken from the same experiments as LOD, LLOQ and ULOQ estimations, providing all required concentrations of HBV DNA are ultimately tested in a manner that will satisfy the requirements for each of the estimations.

The test results will 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.

Commercial HBV DNA panels containing HBV genotypes at high viral loads may be used as parent material.

<table>
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<th>CLSI EP06-A (20)</th>
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### 1.8 Validation of the assay procedure

#### 1.8.1 Validation of primer and probe choice

For each claimed genotype, evidence supporting the choice of critical reagents (primers and probe sequences) shall be provided.

Evidence shall include:

- Rationale for selection of primers and probes including specific sequences used.
- Justification for alignments made to generate consensus sequences or best-fit modifications made to existent sequences e.g., to permit maximum homology to several strains, and
- Information on size, GC content, melting temperatures, hairpin or other secondary structures if any, and the nucleotide position on the genome map of the primers and probes.

For assays designed to detect or quantitate multiple HBV genotypes, data should be provided to demonstrate that the primers and/or probes chosen are effective for all genotypes claimed.

| IMDRF IVD MA ToC (6) |

#### 1.8.2 Whole system failure rate

The potential for false negative results in low positive specimens shall be determined:

- The specimen panel shall be randomised and will contain 20 contrived weak positive specimens (3 x LLOQ) (see note 2).

This may be conducted as a part of precision studies (section 1.4.2) if the minimum number of replicates are met (see chapter 4.3).

Replicate contrived specimens should be prepared using a single genotype specimen diluted in the appropriate matrix.
2. The panel shall be tested:
   - On 5 consecutive days (to give a total of 100 test results)
   - Using 1 lot
   - With 1 user
3. The whole system failure shall be determined in the most viscous specimen type claimed, e.g., whole blood

### 1.8.3 Carry-over contamination

1. The potential for carry-over contamination or similar shall be investigated using a panel of 40 alternating high positive (≥ $10^8$ IU/mL) and negative specimens
2. Only one claimed genotype is required
3. Only one specimen type should be used. The specimen type with the highest viscosity (e.g., whole blood versus plasma) shall be chosen as the test specimen. In addition, DBS specimens should also be tested if claimed in the IFU (see note 1)
4. The panel shall be tested:
   - In at least 5 different runs
   - On 3 different days
   - At least 2 users
   - Using 1 lot
5. For testing platforms that can only accommodate a single specimen, testing shall be conducted on a single instrument:
   - At least 4 tests per run
   - Using alternating high-positive (≥ $10^8$ IU/mL) and negative specimens
   - A total of 10 runs
   - By at least 2 users
   - Using 1 lot

### 1.9 Software

1. Software validation (including verification of built-in fail-safe and alert mechanisms)

### 1.10 Usability/human factors

1. If software is utilized for amplification, detection, and calculation of quantitative or qualitative results, validation of such software for the intended function should be provided

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Haeckel R (21)

FDA (23)
1. Evidence is required to demonstrate that the conditions recommended in the IFU are validated and how they were verified.

2. The influence of the following factors on expected results (both detected and non-detected) shall be considered as applicable (see note 1):
   - Specimen and/or reagent volume
   - IVD instrument sturdiness (including the effect of non-level work surface)
   - Lighting, humidity and barometric pressure (simulating high altitude)
   - Handling contamination (e.g., from latex, powder, hand lotion, sweat, and/or soap, etc. as appropriate)
   - Operating temperature

3. Instrumentation (both extraction and amplification) including:
   - Ruggedness (including the effect of vibration from other instruments) (see note 4)
   - Impact of dust and mould on componentry (e.g., optics)
   - Impact of power/voltage fluctuations

4. Where different specimen types are claimed, flex studies shall use the most challenging specimen type (for example, whole blood)

5. Studies investigating the impact of specimen volume/specimen adequacy (e.g., DBS) shall be conducted in all specimen types

6. Testing to be performed in 1 lot

7. The specimen panel shall contain:
   - 1 negative specimen
   - 1 low positive specimen (3 x LLOQ)

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1. Refer to WHO document PQDx_018 “Instructions for compilation of a product dossier” for other flex studies that may be relevant, taking into consideration the broad range of operational and environmental conditions consistent with intended use in resource limited settings.

2. 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 instructions for use, temperature ranges should be investigated that exceed those of claimed operating conditions and which could cause test failure (incorrect/invalid results).

3. Additional factors may be relevant for POC devices or devices requiring significant manual interventions (e.g., manual DBS extraction). These factors may include errors during sample collection, sample handling and loading, and handling of relevant test components after sample application.

4. For the purposes of this document, ruggedness means the ability to resist environmental shocks of a variety of kinds.

5. Robustness testing generally takes the form of statistically designed experiments to evaluate the effect of simultaneous “small but deliberate changes” in method parameters.

6. Since assay and analyser parameters are locked down in a closed system and cannot be changed, there should be evidence that these parameters have been optimized.

7. The manufacturer shall define what the acceptable deviation is.
### 1.11 Stability of the IVD

#### 1.11.1 General requirements

1. Testing shall be undertaken using a stability testing panel consisting of at least (see note 2):
   - 1 negative specimen
   - 1 low positive specimen (3 x LLOQ)
   - 1 medium positive
   - At least 3 replicates of each panel member shall be tested at each time point (see note 3)
   - Only 1 genotype is required
2. If the assay contains more than one of each primer/probe combination, then each primer/probe combination needs to be assessed for stability

#### 1.11.2 Claimed shelf-life including shipping stability

Stability studies shall be evaluated for the shelf life of the IVD.
1. The following conditions shall be investigated:
   - Conditions to mimic extremes of conditions (temperature, humidity, pressure) exposed to during transport (see note 4)
   - Storage temperature and humidity range
2. Testing in a minimum of 3 lots (see note 5)
3. All claimed specimen types shall be tested. If both venous and capillary whole blood is claimed, then only one of these specimen types need to be assessed. If equivalence between claimed anticoagulants has been demonstrated, only 1 anticoagulant is required to be tested.
4. Multiple instruments may be used to allow simultaneous testing at each time point

#### 1.11.3 In-use stability

1. In-use stability shall be evaluated in at least 1 lot
2. Testing shall be conducted using the most challenging specimen type
3. All labile components (e.g., buffers vials, sealed cartridges, etc.) shall be evaluated
4. In-use stability of labile components shall be conducted using components in their final configuration

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1. When more than one part of the genome is targeted by primers, each region shall be monitored separately during stability evaluation
2. Specimens with target levels of analyte can be contrived; in other words, manufactured by spiking an appropriate negative matrix with HBV DNA positive material with a known concentration HBV DNA
3. Justification for the number of replicates shall be based on the stability study set up, statistical analysis of the data and a prior knowledge of the assay’s performance
4. Determination of shipping stability shall be performed using simulated extreme stress conditions, ensuring that application of those conditions is consistent and controlled. Transport stress shall be applied before assigning lots to shelf-life studies to mimic the real-life situation
5. Lots defined as per chapter 4.3 of this document
6. The number of invalid tests with each kit lot shall be reported
7. Statistically designed experiments should be involved to allow evaluation of any interactions between environmental conditions
8. The acceptance criteria shall be stringent enough to highlight changes over time
9. 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 were demonstrated at 15 months, then the maximum stability claim shall be 12 months
10. Accelerated studies do not replace the need for real time data for shelf-life and in-use stability

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ISO 23640 (24)  
CLSI EP25 (25)  
TGS-2 (26)  
ASTM D4169-14 (27)
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<td>5.</td>
<td>On-board stability shall be tested for an IVD used with an instrument</td>
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Part 2: Clinical evidence (clinical performance characteristics)

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<th>Notes on testing requirements</th>
<th>Source documents</th>
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<tr>
<td>2.1 Clinical sensitivity and specificity</td>
<td>Studies shall be designed to evaluate the intended use(s) of the IVD</td>
<td>1. Clinical performance shall be established using specimens that correspond directly to claims made in the IFU 2. The comparator method shall be a state-of-the art HBV NAT assay as described in chapter 4.3 of this document 3. Clinical performance study protocols shall specify how results in the IVD under evaluation and the reference method will be compared and how results in the 2 assays will be statistically determined to be equivalent or not (e.g., Bland Altman analysis for quantitative IVDs) 4. Problematic specimens, those with unexpected results but which otherwise meet selection criteria for a study, shall not be systematically excluded from analysis 5. 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 6. All invalid test results shall be recorded. Invalid results should be reported as individual categories (e.g., internal control failure, extraction failure, etc.) and not aggregated. Invalid results should be analysed separately in the final performance calculations 7. For IVDs that claim use for clinical management of chronic HBV infection, the positive predictive value (PPV), negative predictive value (NPV) and odds ratio (OR) of the biochemical and HBeAg loss responses from each test individual should be calculated with respect to the virological response. Histologic response may also be evaluated 8. Up to 25% of the test specimens may be well-characterised stored specimens assuming that freezing specimens has been validated during analytical performance studies (section 1.1.1). If the clinical</td>
<td>CLSI EP09-A3 (12) WHO (28) Commission implementing regulation (EU) 2022/1107 (15)</td>
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<td>2.1.1 General requirements for clinical sensitivity and specificity studies</td>
<td>Testing shall be conducted: 1. In specimens from different geographical settings (minimum of 2 regions that represent the diversity of the intended test population and the genetic variability) (see note 1) 2. By a variety of intended users representative of different intended test settings (e.g., primary, provincial) 3. For each claimed specimen type 4. Specimens shall be collected from the intended use population 5. Using at least 2 lots (see chapter 4.3 of this document) 6. Specimens shall be tested from individuals infected with all the common genotypes found as well as any other claimed genotypes 7. The specimens shall also be tested by the reference assay (see notes 2, 3) 8. Discrepant or unexpected results shall be fully evaluated (see note 3, 4, 5, 6) 9. The procedure for selection of study specimens, how these represent the intended population and how bias has been addressed shall be clearly described</td>
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<td>2.1.2 Clinical sensitivity &amp; specificity</td>
<td>1. Testing shall be conducted using both IVD and reference assays on specimens from at least 300 HBV-infected individuals who are enrolled on anti-viral treatment 2. Testing of specimens from each enrolled individual shall be conducted at the following time points: • Baseline specimens (i.e., before treatment)</td>
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<td>3.</td>
<td>At 12, 24 and 48 weeks of treatment</td>
<td>study uses higher number of stored specimens, please discuss this with WHO in advance of submission</td>
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<td>3.</td>
<td>Biochemical (e.g., ALT) and HBV serological testing (e.g., HBeAg, anti-HBe, HBsAg, anti-HBsAg) of each specimen at every time point should be conducted (see note 7)</td>
<td>9. The clinical performance estimate shall be each specimen type and not for aggregated data</td>
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<td>4.</td>
<td>Histologic testing may also be done for each individual (see note 7)</td>
<td>10. Estimate of clinical sensitivity and specificity shall be reported with 95% confidence intervals</td>
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<td>5.</td>
<td>Testing of specimens from at least 100 individuals known not to be infected with HBV shall be conducted for each specimen type</td>
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### Part 3: Qualification of usability for near POC testing by healthcare professionals

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<th>Testing requirements</th>
<th>Notes on testing requirements</th>
<th>Source documents</th>
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<tr>
<td><strong>3.1 Qualification of usability for near POC testing by healthcare professionals</strong></td>
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<tr>
<td><strong>3.1.1 Label comprehension (including IFU)</strong></td>
<td>1. Testing of subjects to assess ability of intended users to correctly comprehend key messages from packaging and labelling that relate to near POC testing: • Understanding key warnings, limitations and/or restrictions, including correct collection methods and equipment • Proper test procedure • Test result interpretation</td>
<td>1. IFU and labelling should be clear and easy to understand. Use of pictorial instructional material is encouraged</td>
<td>IEC 62366-1:2015 (22) Backinger CL and Kingsley PA (29) European Parliament IVD regulations (30)</td>
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<td>2. Studies shall include at least 15 intended users, users including those whose native language may not be the language of the IFU if necessary, to demonstrate comprehension of key messages in the user population</td>
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<td><strong>3.1.2 Results interpretation</strong></td>
<td>1. For near POC tests, intended users shall interpret the results of contrived IVDs to assess their ability to correctly interpret predetermined test results and error messages. 2. Testing subjects to consist of at least 15 intended users including those whose native language may not be the language of the IFU if necessary, from at least two geographically diverse populations to demonstrate correct interpretation of simulated test results and error messages</td>
<td>1. Study group may include subjects recruited as part of the label comprehension study</td>
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</table>
6. Source documents


23. Guidance for Industry In the Manufacture and Clinical Evaluation of In Vitro Tests to Detect Nucleic Acid Sequences of Human Immunodeficiency Viruses Types 1 and 2; FDA, 1999;


29. Backinger CL and Kingsley PA. Write It Right: Recommendations for Developing User Instructions for Medical Devices Used in Home Health Care. HHS Publication FDA 93-4258. Silver Spring, MD: Food and Drug Administration; 1993 Available at: