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 nucleic acid (DRAFT)

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1 Joined by teleconference
2 Joined by teleconference
Abbreviations

ALT alanine transaminase
anti-HBe antibody to hepatitis Be antigen
anti-HBsAg antibody to hepatitis B surface antigen
ANOVA analysis of variance
CI confidence interval
DNA deoxy ribonucleic acid
GHTF Global Harmonization Task Force
HBV hepatitis B virus
HBeAg hepatitis B early antigen
HBsAg hepatitis B surface antigen
IFU instructions for use
IMDRF International Medical Device Regulators Forum
IS International Standard
IVD in vitro diagnostic
IU international units
LOD limit of detection
LLOQ lower limit of quantification
NAT nucleic acid amplification technology
NPV negative predictive value
OR odds ratio
POC point of care
PPV positive predictive value
TGS Technical Guidance Series
TSS Technical Specifications Series
ULOQ upper limit of quantification
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 of tests for the quantification of hepatitis B virus (HBV) deoxy ribonucleic acid (DNA) using nucleic acid amplification techniques (NAT). 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 a suggested method to undertake the testing, but it is not a requirement.

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

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.

Note: For assays that use different combinations of extraction methods/kits and
amplification/detection kits, each combination shall be completely validated as described
in this document.

B. How to apply these specifications

For purposes of WHO prequalification HBV assays with a claim to determine viral load
(quantitative tests) shall comply with the specifications in Part 1 and Part 2 of this
document. Part 3 only applies if, according to the instructions for use (IFU), testing is
performed by non-laboratory professionals in point of care\(^3\) (POC) or near POC settings.

C. Other guidance documents

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

WHO prequalification documents\(^4\)

- Technical Guidance Series for WHO Prequalification - Diagnostic Assessment
- Sample Product Dossiers for WHO Prequalification - Diagnostic Assessment
- 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;\(^5\)
- Guidelines on hepatitis B and C testing;\(^6\)
- Guidelines on hepatitis B and C testing - Policy brief\(^7\).

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\(^3\) Point-of-care (POC) in-vitro diagnostic testing 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


\(^5\) [https://apps.who.int/iris/bitstream/handle/10665/154590/9789241549059_eng.pdf?jsessionid=A9EBA3A9E84B4665CA54C8FED2112AF2?sequence=1](https://apps.who.int/iris/bitstream/handle/10665/154590/9789241549059_eng.pdf?jsessionid=A9EBA3A9E84B4665CA54C8FED2112AF2?sequence=1)

\(^6\) [http://apps.who.int/iris/bitstream/handle/10665/254621/9789241549981-eng.pdf?sequence=1](http://apps.who.int/iris/bitstream/handle/10665/254621/9789241549981-eng.pdf?sequence=1)

\(^7\) [http://apps.who.int/iris/bitstream/handle/10665/251330/WHO-HIV-2016.23-eng.pdf?sequence=1&isAllowed=y](http://apps.who.int/iris/bitstream/handle/10665/251330/WHO-HIV-2016.23-eng.pdf?sequence=1&isAllowed=y)
D. Performance principles for WHO prequalification

D.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 detected (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);
- The result output;
- The testing population for which the functions are intended (e.g. see WHO Global Hepatitis programme guidelines);
- The intended operational setting (e.g. laboratory setting, and/or near-POC);
- The intended user;
- The intended specimen type, or methods; and
- Any limitations to the intended use e.g. not intended for blood screening.

D.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.

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 professionals\(^8\) either in centralised testing laboratories or at near-POC,
- laboratory professionals in health care settings not experienced in nucleic acid testing.

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, but also the likely operational settings in which testing will occur, and the genetic variability of hepatitis B in the intended test populations.

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 and test population.

Laboratory demonstration of equivalence between specimen types without evidence of clinical validation is insufficient. For example, studies that comprise the testing of left-over/repository specimens by research and development staff at a manufacturer’s facility shall not, on their own, be considered sufficient to meet many of the clinical performance study requirements summarized in this document.

D.3 Applicability of supporting evidence to IVD under review

Analytical and clinical 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

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\(^8\) Medical technologists, medical laboratory technicians or similar, who have received a formal professional or paraprofessional certification or tertiary education degree.
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). 9 If the protocol section of the IFU has been changed in any way, both the protocol provided to laboratory for 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 are 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. Differences found between lots during the analytical and clinical performance studies shall be reported. Where the manufacturer supplies the instrumentation required to conduct the assay, (e.g. a closed system), 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. For WHO purposes this should be to a standard 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 95% confidence interval around the estimated values for key performance metrics, as appropriate. The cause of invalid results should be reported if available (such as sample issues (e.g. age of sample, storage conditions, inadequate sample volume, instrument error, operator error). Data should be presented in a clear and understandable format.

It is unlikely that clinical specimens will be available in the volumes required for all analytical studies. 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.

9 http://apps.who.int/iris/bitstream/handle/10665/251915/WHO-EMP-RHT-PQT-2016.01-eng.pdf;jsessionid=30D5BF0B09FFDA38BA1698E65C8B496?sequence=1
For certain analytical 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.

If the validation of specimens (section 1.2.1) shows equivalency between specimen types, some studies, as indicated in this document, may require validation in one specimen type only. However, if no equivalence between specimen types is shown, validation shall be conducted in all specimen types in those studies indicated in the TSS.

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 (including age of the specimen);
- the stability of the nucleic acid target.

Clinical studies shall be based on testing human specimens only sourced from population cohorts reflective of the intended use.
### PART 1
#### ANALYTICAL PERFORMANCE AND OTHER EVIDENCE

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### PART 2
#### CLINICAL EVIDENCE

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### Part 1: Analytical performance and other evidence

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| **1.1 Stability of sample(s)**              | **1.** Real time studies shall be determined 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 (duration at different temperatures, temperature limits, humidity freeze/thaw cycles)  
• intended use (see note 1)  
**2.** A minimum of 10 weak positive specimens (approx. 3 x LLOQ) shall be used (see notes 2):  
• the panel shall include different genotypes | 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 spiking the appropriate negative 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.  
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. If dried blood spots are a claimed specimen type, the details of the filter paper (brand, product code) shall be specified and the use and stability validated.  
6. 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 | TGS 3 (1) |
| **1.2 Validation of specimens**             | **1.2.1 Demonstration of validity of 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 notes 1-3)  
**2.** 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 | 1. 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.  
2. Similarly, if equivalence is claimed in specimens collected into multiple anticoagulants, each specimen shall be collected into each of the claimed anticoagulants. | IMDRF MA TOC (2) |
### 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) | 1. The version of the international standard used shall be stated. |
| | 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 that has been stringently assessed and authorized for use by a regulatory authority of the founding members of GHTF | 2. The IVD should provide accurate external low positive, high positive, and negative controls that are processed along with specimens through all steps of the procedure. External controls are provided either by the manufacturer or are obtained from an independent source. Such controls are processed as normal test samples. A negative control is a sample of a suitable matrix shown to be negative for the target. |
| | | 3. 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. |
| | | 4. An internal control shall be added to each sample 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 |

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10 Global Harmonization Task Force founding members are Europe, USA, Australia, Canada and Japan.
Analytical performance

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<td></td>
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<td>non-target sequence of the same type of nucleic acid (DNA) as the target, which are extracted and amplified simultaneously with the target sequence (in the same well/vial). Therefore, the test should be able to clearly identify the amplified products (amplicons) of the internal control and the target. Some quantitative assays may include an internal calibrator which is assayed simultaneously with the target to allow for quantification of specimens based on algorithms that compare signal generated from the internal calibrator and target sequence to a pre-generated standard curve.</td>
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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 comparator method (see note 1)

2. The following specimens shall be tested by the IVD and the comparator method:
   - at least 100 specimens of each claimed specimen type positive for HBV DNA (see note 2)
   - 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 two lots shall be used for the testing.

1. The comparator method shall be authorized for use by a regulatory authority of the founding members of GHTF 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.

3. Either contrived specimens with varying viral loads or clinical specimens may be used.

4. Correlation of results between the IVD and the comparator method shall be demonstrated statistically.

5. Trueness may be established during the clinical study for diagnostic sensitivity and specificity (section 2.1).

CLSI EP09-A3 (6)
CLSI EP15-A3 (7)

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:
   - 1 x negative specimen
   - 1 x low positive specimens (approx. 3 x LLOQ) (see note 3)
   - 2 x medium positive specimens (see notes 3 & 4)

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 (1)
CLSI EP05-A3 (8)
CLSI EP12-A2 (9)
## Analytical performance

### Technical Specifications for submission to WHO prequalification - Diagnostic Assessment:
IVDs used for the quantitative detection of HBV DNA

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<td>4. The concentrations of DNA for the medium and high positive specimens should span the linear range of the assay, including the upper limit of quantification.</td>
<td>TSS 15</td>
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<td>5. The testing panel should be the same for all operators, lots and sites.</td>
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<td>6. 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.</td>
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<td>7. Each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents, representative of routine manufacture.</td>
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<td>8. The number of invalid tests shall be reported.</td>
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<td>9. Results shall be statistically analyzed (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.</td>
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<td>10. To understand irregularities in results obtained, at least 2 lots should be tested at each of the 3 testing sites.</td>
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<td>11. Alternative methods used to establish repeatability and reproducibility performance of the assay shall be discussed with WHO in advance of dossier submission.</td>
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### 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 (3 x 8) replicate tests of a minimum 8-member 0.5 log_{10} dilution panel of a suitable biological reference material (e.g. WHO HBV DNA IS or a secondary standard calibrated against it)

2. The replicate testing shall be conducted on 3 different days (see note 2)

3. Positive specimens in the panel shall all be of the same genotype

4. Each panel member shall be tested:
   - in 5 replicates
   - using 3 different lots (see notes 7 & 10)
   - over 5 days (not necessarily consecutive) with one run in that day (alternating morning/afternoon)
   - at each of 3 different testing sites (see note 5)
   - by 1 operator/site (see note 6)
   - by personnel representative of intended users
   - unassisted
   - using only those materials provided with the IVD (e.g. IFU, labels and other instructional material)
   - All claimed specimen types shall be tested

4. LOD for HBV DNA shall be expressed in international units with a 95% confidence interval that takes into account lot to lot variation. The 95% confidence interval will be that obtained by analysis of combined data from both lots.

5. An LOD is determined for each of two lot numbers separately; the higher of these two determinations is reported as the LOD for the assay.

6. For low throughput instruments, the number of testing days may be increased.

7. CLSI MM06-A2 (4)

8. European Commission decision on CTS (10)

9. CLSI EP12-A2 (9)

10. CLSI EP17-A2 (11)
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| 1.5.2 Limits of quantification | Lower and upper limits of quantification (LLOQ, ULOQ) 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 x 5) 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.  
2. For the ULOQ, determination, a dilution series prepared from a high-titre clinical specimen isolate shall be tested in a similar manner  
3. The concentrations of the dilution panel members shall go beyond the claimed LLOQ and ULOQ  
4. LLOQ shall be estimated for each claimed genotype  
5. LLOQ shall be estimated for each claimed specimen type  
6. The replicate testing shall be conducted on three different days  
7. Using 2 different lots (see chapter D.3) | 1. The version of the IS used shall be stated.  
2. Predefined criteria for acceptable accuracy (precision & trueness) at the LLOQ and the ULOQ shall be provided.  
3. ULOQ should be estimated for each specimen type | European Pharmacopoeia (5)  
CLSI EP05-A3 (7)  
European Commission decision on CTS (10)  
CLSI EP17-A2 (11)  
PQDx_018 (12) |
| 1.6 Analytical specificity     | The potential for false results (false nonreactive and false reactive results or inaccurate results) arising from interference from at least the substances/conditions (see note 1) listed below shall be determined. by testing confirmed HBV DNA-negative samples, both spiked (using a single, common HBV genotype) and unspiked: | 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. | European Commission decision on CTS (10)  
CLSI EP07 (13)  
CLSI EP37 (14) |
## Analytical performance

### Technical Specifications for submission to WHO prequalification-Diagnostic Assessment: IVDs used for the quantitative detection of HBV DNA

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|                 | 1. Substances/conditions represented, where possible, by at least 5–10 specimens from different individuals.  
2. spiking with HBV at approx. 3 x LLOQ  
3. using only one claimed specimen type | 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 (approximately $10^4$ to $10^5$ IU/mL if possible).  
5. 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.  
6. 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.  
7. 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.  
8. 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.  
9. Any observed cross-reactivity shall be further investigated and performance limitations of the IVD reported in the IFU. |                                                                                                      |
| 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. Haemoglobin, lipids, bilirubin, albumin  
2. Elevated immunoglobulin concentrations  
3. Elevated liver enzymes such as ALT, AST, GGT  
4. Alcoholic cirrhosis  
5. 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, such as (see note 5):  
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 medicines  
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  
4. Medicines or biologicals that increase circulating nucleic acid  
5. Presence of nucleic acid-based medicines and metabolites and binding substances |                                                                                                      |
| 1.6.2 | 1. The potential for false positive results shall be determined arising from cross-reactivity (see note 1) with other organisms |                                                                                                      |
### Cross-reactivity

Unrelated to hepatitis B or disease states commonly found in regions of the intended use of the IVD, including where possible, at least 5–10 of the commonly found DNA viruses such as (see note 9):

**Viruses, such as:**
- HIV 1 & 2
- Hepatitis A, C, D 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 exceed the expected upper and lower limits of the measuring range shall be tested
2. At least 3 replicates tested at each concentration

1. 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.

CLSI EP06-A (15)
### Analytical performance

#### Technical Specifications for submission to WHO prequalification—Diagnostic Assessment:

**IVDs used for the quantitative detection of HBV DNA**

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<td>3.</td>
<td>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)</td>
<td>2. 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.</td>
</tr>
<tr>
<td>4.</td>
<td>Using 1 lot</td>
<td>3. Commercial HBV DNA panels containing HBV genotypes at high viral loads may be used as parent material.</td>
</tr>
<tr>
<td>5.</td>
<td>All claimed specimen types shall be tested</td>
<td></td>
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<tr>
<td>6.</td>
<td>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</td>
<td></td>
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</tbody>
</table>

#### 1.8 Validation of the assay procedure

<table>
<thead>
<tr>
<th>1.8.1 Validation of primer and probe choice</th>
<th>For each claimed genotype, evidence supporting the choice of critical reagents (primers and probe sequences) shall be provided</th>
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<tbody>
<tr>
<td>1. Evidence shall include a</td>
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<tr>
<td>• rationale for selection of primers and probes including specific sequences used</td>
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<tr>
<td>• 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</td>
<td></td>
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<tr>
<td>• 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.</td>
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<tr>
<td>2. 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.</td>
<td></td>
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<tr>
<th>1.8.2 Whole system failure rate</th>
<th>The potential for false negative results in low positive specimens shall be determined:</th>
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<tbody>
<tr>
<td>1. The specimen panel shall be randomised and will contain 20 contrived weak positive specimens (3 x LLOQ) (see note 2)</td>
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<tr>
<td>2. The panel shall be tested:</td>
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<tr>
<td>• on 5 consecutive days (to give a total of 100 test results)</td>
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<tr>
<td>• using 1 lot</td>
<td></td>
</tr>
<tr>
<td>• with 1 user</td>
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</table>

1. This may be conducted as a part of precision studies (section 1.4.2) if the minimum number of replicates are met (see chapter D.3). |
2. Replicate contrived specimens should be prepared using a single genotype specimen diluted in the appropriate matrix. |
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<tr>
<td><strong>3.</strong> The whole system failure shall be determined in the most viscous specimen type claimed, e.g. whole blood.</td>
<td></td>
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</tbody>
</table>
| **1.8.3 Carry-over contamination** | 1. The potential for carry-over contamination or similar shall be investigated using a panel of 40 high positive ($\geq 10^8$ IU/mL) and 40 negative specimens (alternating)  
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 ($\geq 10^8$ IU/mL) and negative specimens  
   - a total of 10 runs  
   - by at least 2 users  
   - using one lot  | 6. If DBS are a claimed specimen type and require punching/excision of the DBS prior to extraction, those procedures shall also be evaluated in the carry-over contamination studies. | Haeckel R (17) |
| **1.9 Usability/human factors** |  |  |  |
| **1.9.1 Flex studies/robustness** | Evidence is required to demonstrate that the conditions recommended in the IFU are validated and how they were verified  
1. The influence of the following factors on expected results (both positive and negative) shall be considered as applicable (see note 1):  
   - Specimen and/or reagent volume | 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 | PQDx_18 (12)  
IEC 62366-1:2015 (16) |
### Analytical performance

#### Technical Specifications for submission to WHO prequalification: Diagnostic Assessment: IVDs used for the quantitative detection of HBV DNA

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<tbody>
<tr>
<td>1.9.2</td>
<td>Software validation</td>
<td>1. <strong>Software validation</strong> (including verification of built-in fail-safe and alert mechanisms)</td>
<td>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.</td>
</tr>
</tbody>
</table>
| 1.10.1 | Shelf-life (including transport stability) | 1. Stability studies shall be evaluated for the shelf life of the IVD. The following conditions shall be investigated: | ISO 23640 (18)  
CLSI EP25 (19)  
TGS-2 (20)  
ASTM D4169-14 (21) |
<p>|        |                      | 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. Lots defined as per chapter D.3 of this document. | |
|        |                      | 4. The number of invalid tests with each kit lot shall be reported. | |</p>
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</table>
| 3.     | The stability panel shall consist of  
• 1 negative specimen  
• 2 low positive specimens (3 x LLOQ)  
• 1 medium positive  
4. Each panel member shall be tested in triplicate at each time point/condition  
5. Only one genotype should be tested  
6. All claimed specimen types shall be tested  
7. Multiple Instruments may be used to allow simultaneous testing at each time point | 5. 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.  
6. Transport stress shall be applied before assigning lots to shelf-life studies to mimic the real-life situation.  
7. Accelerated studies do not replace the need for real time data for shelf-life and in-use stability. | |
| 1.10.2 | In-use stability (open pack or open vial stability) | 1. Most aspects of in use stability may be included as part of section 1.10.1 (robustness studies). | |
| 1.     | Testing of a minimum of 1 lot using a panel composed of:  
• 1 negative specimen  
• 2 low positive specimen (approx. 3 x LLOQ)  
2. Each panel member shall be tested in triplicate  
3. Only 1 claimed specimen type should be tested  
4. All labile components (e.g. buffers vials, sealed cartridges, etc.; see note 3) shall be evaluated  
5. On-board stability shall be tested for an IVD used with an instrument | | |
## Part 2: Clinical evidence

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<tr>
<td>2.1 Diagnostic sensitivity and specificity</td>
<td>Studies shall be designed to evaluate the intended use(s) of the IVD. 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 note 3) 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 comparator assay (see notes 4 &amp; 5) 8. discrepant or unexpected results shall be fully evaluated (see note 2) 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>
<td>1. Clinical performance shall be established using specimens that correspond directly to claims made in the IFU. 2. Problematic specimens, those with unexpected results but which otherwise meet selection criteria for a study, shall not be systematically excluded from analysis. 3. Lots will be selected in line with specifications in chapter D.3 of this document. 4. The comparator method shall be a state-of-the-art HBV NAT assay as described in chapter D.3 of this document. 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.</td>
<td>CLSI EP09-A3 (6) WHO (22)</td>
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### 2.1.1 General requirements for clinical evaluation studies

- Baseline specimens (i.e. before treatment)
- At 12, 24 and 48 weeks of treatment

### 2.1.2 Diagnostic sensitivity & specificity

- 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.

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European Commission decision on CTS (10)
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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 1)</td>
<td>Up to 25% of the test specimens may be well-characterised stored specimens assuming that freezing specimens has been validated during analytical studies (section 1.1.1).</td>
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<td>4.</td>
<td>Histologic testing may also be done for each individual (see note 1)</td>
<td>Clinical performance study protocols shall specify how results in 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 for quantitative IVDs).</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 (see notes 3-4)</td>
<td>The clinical performance estimate shall be each specimen type and not for aggregated data.</td>
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<td>Estimate of diagnostic specificity shall be reported with 95% confidence intervals.</td>
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</table>
F. References

   http://apps.who.int/iris/bitstream/10665/258985/1/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf?ua=1

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   http://www.who.int/entity/diagnostics_laboratory/evaluations/141015_pqdx_018_dossier_instructions_v4.pdf?ua=1


