WHO Performance Evaluation Acceptance Criteria for HBsAg In vitro diagnostics in the context of WHO Prequalification

In primary Hepatitis B virus (HBV) infection, Hepatitis B surface antigen (HBsAg) can be detected in the blood after an incubation period ranging from a few days to up to 10 weeks, followed shortly by development of antibodies against the HBV core antigen (anti-HBc antibodies). The viremia is well established by the time HBsAg is detected in blood, and titres of virus in acute infection are very high (10^9 to 10^10 virions per millilitre). In persistent HBV infection, HBsAg remains in the blood and viral replication continues, often for life. However, levels of viremia in active infection are generally substantially lower than during primary infection, although the levels can vary considerably from person to person. The presence of HBsAg indicates that the person is potentially infectious.

HBsAg assays can be used for screening of blood for transfusion, testing of asymptomatic individuals for occult disease and to confirm diagnosis in symptomatic patients suspected of having HBV infection. The performance requirements for these testing purposes should correspond to expected HBsAg levels in the different patient populations. Screening of blood for transfusion would require assays with better analytical sensitivity to ensure detection of the potentially low HBsAg levels such as those found in early seroconversion, in order to minimize likelihood of direct transmission of infection. On the other hand, in individuals suspected of having HBV infection, whether they are asymptomatic or symptomatic, generally, the levels of HBsAg are generally high and thus the need for superior analytical sensitivity in an assay is not as critical.\(^1\)

A review of the literature and data generated from previous WHO performance evaluations indicate significant differences in the performance of HBsAg enzyme immunoassays (EIAs) and rapid diagnostic tests (RDTs). Data from these studies show that the analytical sensitivity of most RDTs is in the range of 2-10 IU/mL which is about 50 to 100 fold less sensitive compared to widely-used CE-marked EIAs. This has prompted WHO to review and develop appropriate performance criteria that can be used for testing of the blood supply and suspected HBV infection, irrespective of symptoms.

The main purpose of the WHO Prequalification (PQ) performance evaluation of HBsAg assays is to assess the performance of the assays in specimen representative of WHO Member States. To achieve this, several specimen panels are used. They include a characterized WHO HBsAg clinical reference panel containing specimens from various

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\(^1\) Jaroszewicz J et al. Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV)-infection: A European perspective. J. Hepatology 2010 vol. 52 j 514–522
geographic regions, a lot-to-lot variation panel, HBsAg seroconversion panels (commercially acquired), HBsAg low titre performance panels (commercially acquired) and the 1st International WHO HBsAg subtype adw2, genotype A Reference Panel (NIBSC code: 03/262). Owing to differences in the circulating HBsAg serotypes, HBsAg assays should have the ability to detect multiple HBsAg serotypes including commonly occurring mutants. As such, future WHO performance laboratory evaluation panels will include HBsAg mutants.

Current WHO procurement eligibility for HBsAg assays requires that assays have a diagnostic sensitivity and specificity of 100% and > 98% respectively for both EIAs and RDTs. Although analytical sensitivity and seroconversion data are collected during the performance evaluation, these results are currently not used in the pass/fail criteria. The European Union Common Technical Specifications (CTS) require all HBsAg assays for clinical diagnosis and blood transfusion screening to have a minimal diagnostic sensitivity equivalent to established screening devices that are CE-marked. For screening of blood donors, the CTS require a diagnostic specificity of 99.5% for all assays except RDTs where the diagnostic specificity required is of at least 99.0%.

The CTS require an analytical sensitivity of <0.130 IU using the 2nd International Standard for Hepatitis B virus surface antigen, subtype adw2, genotype A (NIBSC code 00/588) for all assays regardless of the format.

The objective of this short guidance developed as a wide collaboration with Hepatitis and regulatory experts is to outline the minimal performance characteristics of HBsAg assays for WHO Prequalification purposes. Figure 1 summarizes the process of performance evaluation of HBsAg In vitro Diagnostic Tests.

All HBsAg assays submitted for WHO PQ will first undergo a stage one performance evaluation to assess the analytical sensitivity [Limit of Detection (LoD)] using the WHO 1st International Reference Preparation for HBsAg. An analytical sensitivity threshold of 4 IU/mL for both EIAs and RDTs will be used as a minimum requirement. Assays found to have a LoD >4 IU/mL, will be considered to have failed to fulfil WHO PQ requirements, it will not be tested further and the PQ process will be cancelled. EIAs and RDTs with a LoD <4 IU/mL will be tested further on the following panels:

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WHO HBsAg clinical specimen reference panel;
Lot-to-lot variation specimen panel (HBsAg positive);
Commercially acquired HBsAg Seroconversion;
Commercially acquired HBsAg low titre performance panel; and
HBsAg mutant panel.

At the end of the performance evaluation there will be two scenarios:

1. Assays for blood screening that have an HBsAg LoD <0.13 IU/mL, diagnostic sensitivity and specificity of 100% and >98% respectively for EIAs and diagnostic sensitivity and specificity of >99% and >98% respectively for RDTs and have satisfied other PQ requirements (product dossier and quality management system (QMS) reviews) will be prequalified and deemed suitable for screening of the blood supply in addition to diagnosis of symptomatic and asymptomatic individuals.

2. An assay with a LoD <4.0 IU/mL, diagnostic sensitivity and specificity of >99% and >98% respectively for RDTs, and sensitivity and specificity of 100% and >98% for EIAs and have satisfied other PQ requirements (product dossier and quality management system reviews) will be prequalified with an intended use restricted to diagnosis of symptomatic and asymptomatic individuals. The manufacturer will be required to include information in the Instructions for use and other related material that use for screening of blood is not supported. Table 1 below summarizes the WHO performance acceptance criteria of HBsAg In vitro Diagnostic Tests.

Table 1. Summary of the new WHO performance acceptance criteria of HBsAg In vitro Diagnostic Tests.

<table>
<thead>
<tr>
<th>Intended use</th>
<th>EIAs</th>
<th>RDTs</th>
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<tbody>
<tr>
<td>Screening of blood donations, <strong>AND</strong> Testing of asymptomatic and symptomatic individuals for diagnostic purposes.</td>
<td>LoD &lt;0.13 IU/mL</td>
<td>LoD &lt;0.13 IU/mL</td>
</tr>
<tr>
<td></td>
<td>Sensitivity 100%</td>
<td>Sensitivity &gt;99%</td>
</tr>
<tr>
<td></td>
<td>Specificity &gt;98%</td>
<td>Specificity &gt;98%</td>
</tr>
<tr>
<td>Screening of blood donations, <strong>AND</strong> Testing of asymptomatic and symptomatic individuals for diagnostic purposes.</td>
<td>LoD &lt;4 IU/mL</td>
<td>LoD &lt;4 IU/mL</td>
</tr>
<tr>
<td></td>
<td>Sensitivity 100%</td>
<td>Sensitivity &gt;99%</td>
</tr>
<tr>
<td></td>
<td>Specificity &gt;98%</td>
<td>Specificity &gt;98%</td>
</tr>
<tr>
<td>Inter-reader variability</td>
<td>N/A</td>
<td>&lt;5%</td>
</tr>
</tbody>
</table>
Screen all assays (EIA and RDT) with 1st International WHO HBsAg subtype adw2, genotype A Reference Panel Catalogue number 03/262

LoD ≤4 IU/mL

Test on WHO HBsAg specimen reference panel, HBsAg positive lot-to-lot variation panel, HBsAg seroconversion panel, HBsAg low titre performance panel, HBsAg mutants panel.

LoD < 0.13 IU/mL
Sensitivity: 100% (EIA) or >99.0% (RDT)
Specificity (EIA or RDT) >98%

Can be used for screening of blood donations and other clinical purposes if it satisfies other PQ requirements (product dossier and QMS)

LoD >4 IU/mL

LoD >4.0 IU/mL
Sensitivity 100% (EIA) or ≥99.0% (RDT)
Specificity (EIA or RDT) >98%

Can be used for screening of symptomatic and non-symptomatic individuals if it satisfies other PQ requirements (product dossier and QMS)

Not recommended for screening of blood donations

Terminate the performance evaluation and cancel PQ process

Figure 1. Summary of WHO HBsAg performance evaluation process.