4.3. How to test HBV

Decision-making tables – PICO 1

HBsAg testing: Among persons identified for hepatitis B testing, what is the diagnostic accuracy of available assays for detecting HBsAg (RDT, EIA)?

**Topic for analysis:** How to test

**Population:** Persons identified for HBV testing

**Intervention:** Rapid diagnostic test for HBsAg detection

**Comparison:** Enzyme immunoassays for HBsAg detection

**Outcomes:** Diagnostic accuracy (Sensitivity, Specificity, Positive predictive value, Negative predictive value, TN, TP, FN, and FP).

**Background:**

- The most important marker for the diagnosis of hepatitis B infection that may require treatment remains the detection of hepatitis B surface antigen (HBsAg).
- Chronic hepatitis B infection is defined by the detection of HBsAg on two occasions six months apart.
- However, after initial testing, further characterization of the individual’s HBV infection is based on a sequential testing strategy of for other markers of HBV infection (supplementary testing) triggered by the detection of HBsAg in the first instance.

**Immunooassays (laboratory-based)**

- The most widely used HBsAg assays are laboratory-based immunoassays.
- This can be in the form of an enzyme immunoassay (EIA), chemiluminescence immunoassay (CLIA) or electrochemiluminescence immunoassay (ECL).
- These are best suited to settings with high throughput of specimens and where infrastructure (electricity, cold storage, climate-controlled rooms) and skilled staff are consistently available.
- Other simple assays such as agglutination assays are also available for detection of HBsAg but these generally require serum/plasma specimens and cold storage. The results of simple assays may be read visually.

**Rapid diagnostic tests (RDTs) – performed in-laboratory or at the point-of-care**

- Many laboratories in resource-limited settings may not have access to specialized equipment and
process few specimens, per day. Hence, individual tests, including rapid diagnostic tests (RDTs), may be more appropriate.

- RDTs for detection of HBsAg come in immunofiltration (flow through) and immunochromatographic (lateral flow) formats.
- In general, RDTs do not require cold storage and may be tested using capillary (finger-stick) whole blood.
- The manufacturer’s instructions for use should always be followed. The results of RDTs are read visually.
- RDTs may be deliverable at the point of care (POC).
- The expansion of their use depends on their performance and operational characteristics in the setting of intended use, ultimately with the aim being to reach resource-limited settings and offer cost-efficient testing services as an alternative to assays that require specific laboratory infrastructure and staff skills to perform.

The selection of EIA or RDTs should not be mutually exclusive. Choice of appropriate technology can be complex but can usually be distilled down to three main factors: performance, cost and accessibility. There are inevitably trade-offs, based not only on disease prevalence and the health-care infrastructure, but also on technical, socioeconomic, cultural, behavioural considerations.

**DRAFT recommendation(s):**

<table>
<thead>
<tr>
<th>Sub-analysis</th>
<th>Study</th>
<th>Pre 2005</th>
<th>19</th>
<th>96.9 (96.0–97.7)</th>
<th>99.7 (99.6–99.8)</th>
<th>266 (106–665)</th>
<th>0.056 (0.033–0.095)</th>
<th>2.72</th>
<th>0.91</th>
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<tbody>
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<tr>
<td></td>
<td>Post 2005</td>
<td>44</td>
<td>86.4 (85.2–87.5)</td>
<td>99.4 (99.2–99.5)</td>
<td>84.6 (43.6–165)</td>
<td>0.126 (0.087–0.183)</td>
<td>4.10</td>
<td>1.27</td>
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<td></td>
<td>Case–control</td>
<td>21</td>
<td>96.7 (96.0–)</td>
<td>99.3 (99.0–)</td>
<td>105 (48.0–)</td>
<td>0.028 (0.010–)</td>
<td>2.23</td>
<td>4.86</td>
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</tr>
</tbody>
</table>

**Summary pooled diagnostic accuracy of rapid HBsAg assays stratified by study, patient, index and reference test**

- High
- Moderate
- Low
- Very low

□ High
□ Moderate
□ Low
□ Very low

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Page | 53
<table>
<thead>
<tr>
<th></th>
<th>Patient Blood donors</th>
<th>HIV+</th>
<th>HIV−</th>
<th>Index Test</th>
<th>Determine</th>
<th>BinaxNOW</th>
<th>VIKIA</th>
<th>Serodia</th>
<th>Reference Test</th>
<th>CMIA</th>
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<td>(90.1–92.9)</td>
<td>(67.9–76.4)</td>
<td>(89.8–94.8)</td>
<td>(89.1–93.9)</td>
<td>(88.9–92.4)</td>
<td>(96.2–98.6)</td>
<td>(77.5–86.7)</td>
<td>(77.5–86.7)</td>
<td>(77.9–82.6)</td>
<td>(77.9–82.6)</td>
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<td>72.3</td>
<td>92.6</td>
<td>91.7</td>
<td>90.8</td>
<td>97.6</td>
<td>82.5</td>
<td>82.5</td>
<td>80.4</td>
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<tr>
<td></td>
<td>(99.3–99.7)</td>
<td>(99.5–99.9)</td>
<td>(99.0–99.9)</td>
<td>(98.9–99.9)</td>
<td>(98.9–99.4)</td>
<td>(99.7–100)</td>
<td>(99.8–100)</td>
<td>(99.8–100)</td>
<td>(99.6–99.3)</td>
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<td></td>
<td>89.2</td>
<td>193</td>
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<td>347</td>
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<td>0.106</td>
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<td>0.077</td>
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<td>0.108</td>
<td>0.045</td>
<td>0.141</td>
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<tr>
<td></td>
<td>(0.055–0.204)</td>
<td>(0.22–0.38)</td>
<td>(0.05–0.13)</td>
<td>(0.058–0.136)</td>
<td>(0.035–0.168)</td>
<td>(0.016–0.128)</td>
<td>(0.026–0.458)</td>
<td>(0.029–0.069)</td>
<td>(0.074–0.268)</td>
<td>(0.074–0.268)</td>
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<tr>
<td></td>
<td>3.82</td>
<td>0.384</td>
<td>2.97</td>
<td>0.81</td>
<td>20.2</td>
<td>3.53</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td>0.44</td>
<td>0.44</td>
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<tr>
<td></td>
<td>1.86</td>
<td>0.0059</td>
<td>0.080</td>
<td>0.24</td>
<td>1.56</td>
<td>1.20</td>
<td>1.472</td>
<td>&lt;0.005</td>
<td>0.73</td>
<td>0.73</td>
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</tbody>
</table>

EIA: enzyme immunoassay; RDT: rapid diagnostic test; CI: confidence interval; *with EIA reference

**Quality of evidence**

*Refer GRADE table in footnote*

**Conclusions:**

- Rapid diagnostic tests, including those performed on whole blood specimens, have good clinical sensitivity and excellent clinical specificity compared to the reference standard (laboratory-based EIA for HBsAg detection). Improvement in both clinical and analytical sensitivity could potentially enhance their impact globally.
  - Caution in HIV-positive individuals is important with significantly reduced clinical sensitivity compared to HIV-negative individuals
  - Reassuring accuracy of whole blood specimens compared to plasma or serum specimens further facilitates use in the field.

**Risks/benefits**

□ Benefits clearly outweigh harms
**Benefits**

**Advantages of testing by RDT compared to laboratory-exclusive EIAs**

- Does not require capital investment in laboratory infrastructure, e.g. EIA plate washers, readers, incubators, analysers, cartridge or random-access analysers
- Concurrent reduction in maintenance costs and reagents
- May be deliverable at the point of care (POC). This may allow greater access to testing and eliminate need for mechanisms for transportation of specimens to the laboratory
- If testing at POC, may reduce number of individuals “lost to follow up”, i.e. never receive their test results
- May be carried out by trained lay providers and health-care workers, in addition to trained laboratory scientists
- Dedicated venepuncture may not be required.

**Risks**

**Disadvantages of testing by RDT compared to laboratory-exclusive EIAs**

- Possible reduction in clinical sensitivity/specificity compared to laboratory-based methods.
- RDTs appear to be less sensitive in HIV-positive individuals.
- Higher cost per test after expense of laboratory infrastructure has been met.
- User variability and subjectivity in reading of a visual assay, second reader suggested.
- Performance characteristics may vary with environmental factors, e.g. heat, humidity, storage conditions.
- Internal quality control measures may be inferior to standardised laboratory assays, e.g. lack of test kit controls, no specimen addition controls.
- Although RDTs using capillary whole blood negate the need for venipuncture and maintenance of laboratory equipment, significant heterogeneity and sub-optimal clinical and analytical sensitivity must be considered.
- Recording of results in a database which can be subsequently interrogated and audited as is the case with centralised laboratory testing may be compromised with testing at POC. This may impact on reporting and epidemiological surveillance of the burden of disease.

**Acceptability, values and preferences**

A values and preferences survey of implementers and users of hepatitis B and
C testing services was carried out by FIND in September 2015. A total of 104 respondents from 43 (20 high-income, 23 low- and middle-income) countries. Relating to this PICO,

- 47% of respondents from low- and middle-income countries would prefer an RDT method of testing using capillary whole blood compared to dedicated venepuncture, even at the cost of reduced clinical sensitivity).
- 50% of respondent would accept an assay with a minimal sensitivity of 95%, 43.5% would accept 98% and 4.3% would accept 90%. However, when the notion of cost was introduced, only 7% responded that 95% sensitivity would not be acceptable.
- 77.3% of respondents preferred results of be available on the same day or sooner. Respondents commented that delay in individuals receiving results was likely to result in a loss to follow-up.

**Community:**

- Support for the most effective testing approach in order to impact on availability of testing, especially in resource-limited settings and remote areas and optimize access to at-risk groups.

**Patients/caretakers:**

- In the setting of HIV, use of RDTs has facilitated scaling up of testing services in terms of widening access to testing services.

**Health-care workers:**

- If RDTs are utilized at the POC, this will allow HCWs to carry out testing and organize follow up potentially in one consultation. There is a need for appropriate training of testing-providers and laboratory staff.
- The intervention was considered likely to be acceptable to key stakeholders as the sensitivity and the specificity of RDT for screening of chronic HBV infections are comparable with EIAs.

**Equity, ethics and human right implications**

*Will the recommendation raise questions around equity?*

- No. The recommendation of the possibility of testing using RDT at POC offers new opportunities for enhancing screening, referral, and treatment for the individuals with chronic HBV infection especially in the resource-limited settings, thus will reduce transmission, morbidity and mortality associated with undetected and untreated HBV infection.
**Are there ethical implications to this recommendation?**

- No major concerns.

**Resource use and financial implications**

**Materials:**

- Cost of test kits
- Cost of sterile lancets, alcohol swabs, gloves, sharps bins or other method of disposal of used kits
- Cost of automated RDT readers, if applicable
- Quality control reagents, if applicable (some kits are supplied with positive and negative test kit controls).

**Training and supervision:**

- Cost of training testing providers and appropriate competency assessment, certification and re-certification of their skills
- From included studies, excellent clinical specificity of all assay formats is reassuring in terms of ensuring cost effective initiation of testing strategies for further investigation and treatment.
- If being utilized at the point-of-care, it will be the responsibility of the testing provider to record and report the result appropriately.

**Other:**

- Creation of a database into which results obtained by POC can be recorded
- Linkage to care, e.g. antenatal clinics

**Possible test procurement cost:**

<table>
<thead>
<tr>
<th>Test</th>
<th>Cost (US$) per test</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDT</td>
<td>0.3–0.95 (procurement cost)</td>
<td>WHO database</td>
</tr>
<tr>
<td>EIA</td>
<td>0.4–2.8 (procurement cost)</td>
<td>WHO database</td>
</tr>
</tbody>
</table>
**Feasibility and constraints to implementation**

Are any major barriers expected for the implementation of this recommendation?

- High-throughput EIAs require certain laboratory infrastructure and equipment with precision and expertise required in its operation.
- Delivery of RDTs requires appropriate training of test providers in performing and reading of the test result, storage of materials and recording and reporting of the status. Decentralisation of testing puts tremendous stress on already fragile health systems in terms of training needs, supply chain management, quality assurance, and monitoring and evaluation of effectiveness and impact. External quality assessment of quality of tests and testing possible but challenging when the need for proficiency panels is increased from a few laboratories to hundreds and possibly thousands of POC sites.

**Feasibility survey report to be presented at meeting.**

<table>
<thead>
<tr>
<th>1.1.10</th>
<th>Is the option feasible to implement?</th>
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<tbody>
<tr>
<td>□ No</td>
<td>□ Probably</td>
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<tr>
<td>□ Uncertain</td>
<td>□ Yes</td>
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<tr>
<td>□ Varies</td>
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</table>

**Relevance to different settings/populations**

Will this recommendation be most relevant for particular settings (e.g. endemi city)?

- The introduction of HBsAg testing using RDTs will be most relevant in settings where there is poor access to existing laboratory testing-services, either access to centralised laboratory testing or lack of testing-infrastructure in existing laboratories.
- Delivery of RDTs at the point-of-care in remote or resource-limited settings, e.g. HBsAg testing in antenatal clinics may significantly affect the future burden of disease.
- Useful for testing of both symptomatic and asymptomatic individuals.
- It will be most relevant to key affected populations who may be at risk of infection but who may be reluctant to or have poor access to health-care services, such as individuals who attend drug-rehabilitation clinics or prisoners. These individuals require screening, may require treatment if infected or vaccination if not currently infected.
- It will be less relevant in individuals who have good access to health care and in settings where laboratory testing for hepatitis B is already well established.

**Rationale for recommendation:**
**Strength of recommendation**

**Implementation considerations**
- Symptomatic vs asymptomatic individuals; in a symptomatic individual, you may not need such good analytical sensitivity than when screening an asymptomatic individual.

**Research gaps**
- Impact of using RDTs for HBsAg at the point-of-care on delivery and implementation of testing services.
GRADE Summary of findings
**Question:** Should RDTs be used to diagnose HBsAg in HIV-negative individuals?

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Studies (N of patients)</th>
<th>Study design</th>
<th>Factors that may decrease quality of evidence</th>
<th>Effect per 1000 patients/year</th>
<th>Test accuracy QoE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Risk of bias</td>
<td>Indirectness</td>
<td>Inconsistency</td>
</tr>
<tr>
<td>True positives (patients with HBsAg)</td>
<td>4 studies 997 patients</td>
<td>cross-sectional (cohort type accuracy study)</td>
<td>serious ¹</td>
<td>not serious ²</td>
<td>serious ³</td>
</tr>
<tr>
<td>False negatives (patients incorrectly classified as not having HBsAg)</td>
<td>4 studies 997 patients</td>
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<td>serious ¹</td>
<td>not serious ²</td>
<td>serious ³</td>
</tr>
</tbody>
</table>

1. Downgraded by one for risk of bias: all studies were prospective cohort studies (1), although one was assessed as high risk of bias because patients were pre-selected based from known chronic hepatitis B patients.
2. Although study was not specifically designed in HIV-negative patients, clear testing and results were included
3. Downgraded by one for inconsistency: unexplained heterogeneity may arise from differences between studies in specimen condition (serum, whole blood), specimen processing (field vs laboratory), reference tests (CMIA; EIA on dried blood spots) and study population (e.g. known chronic hepatitis B patients, general community screen)
4. Downgraded by one for imprecision: confidence intervals extend below 90% accuracy, with tau-squared for PLR >1 (indicating substantial heterogeneity)
**Question:** Should RDTs be used to diagnose HBsAg in HIV-positive individuals?

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>0.72 (95% CI: 0.68–0.76)</th>
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<tbody>
<tr>
<td>Specificity</td>
<td>1.00 (95% CI: 0.99–1.00)</td>
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</table>

| Prevalence | 5% | 20% |

<table>
<thead>
<tr>
<th>Outcome</th>
<th>No. of studies (No of patients)</th>
<th>Study design</th>
<th>Factors that may decrease quality of evidence</th>
<th>Effect per 1000 patients/year</th>
<th>Test accuracy QoE</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Risk of bias</td>
<td>Indirectness</td>
<td>Inconsistency</td>
</tr>
</tbody>
</table>
| True positives (patients with HBsAg) | 5 studies 2566 patients | cross-sectional (cohort type accuracy study) | serious¹ | not serious² | serious³ | serious⁴ | none | 36 (34–38) | 145 (136–153) | ☐☐☐☐
| False negatives (patients incorrectly classified as not having HBsAg) | | | | | | | | 14 (12–16) | 55 (47–64) |
| True negatives (patients without HBsAg) | 5 studies 2566 patients | cross-sectional (cohort type accuracy study) | serious¹ | not serious² | not serious⁵ | not serious⁶ | none | 948 (945–949) | 798 (796–799) | ☒☒☒
| False positives (patients incorrectly classified as having HBsAg) | | | | | | | | 2 (1–5) | 2 (1–4) |

1. Downgraded for one for risk of bias: all studies were prospective cohort studies of consecutive patients. Studies used different specimens (serum, 2; capillary whole blood, 1; venous whole blood, 1), reference standards (CMIA, EIA confirmed by neutralization), and had patients with different ART status (four studies ART naive).
2. Not downgraded for indirectness: all studies performed in cohorts of consecutive patients in Tanzania (2), Ghana (3), Malawi (4), South Africa (5) and Bissau (6).
3. Downgraded by one for inconsistency with sensitivities ranging from 62% to 100%; unexplained heterogeneity may arise from differences between studies in specimen type, specimen processing and study population. Two studies had very high sensitivities (100%, 96%) while the remainder (3,5,6) had low sensitivities (range 62–70%). Tau-squared <1 for studies

4. Downgraded by one for imprecision: confidence intervals 67.9–76.4%. Two studies had very high sensitivities (100%, 96%) while the remainder (3,5,6) had low sensitivities (range 62–70%).

5. Not downgraded for inconsistency: specificities ranged from 99% to 100%, with tau-squared <1

6. Not downgraded for imprecision: narrow confidence interval

**Question:** Should Determine HBsAg be used to diagnose HBsAg in a global setting?

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>0.91 (95% CI: 0.89–0.92)</th>
<th>Specificity</th>
<th>0.99 (95% CI: 0.99–0.99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalences</td>
<td></td>
<td></td>
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<tr>
<td>5%</td>
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<td>20%</td>
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<table>
<thead>
<tr>
<th>Outcome</th>
<th>No. of studies (No. of patients)</th>
<th>Study design</th>
<th>Factors that may decrease quality of evidence</th>
<th>Effect per 1000 patients/year</th>
<th>Test accuracy QoE</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Risk of bias</td>
<td>Indirectness</td>
<td>Inconsistency</td>
</tr>
<tr>
<td>True positives (patients with HBsAg)</td>
<td>12 studies 7552 patients</td>
<td>cohort and case–control type studies</td>
<td>serious</td>
<td>not serious</td>
<td>very serious</td>
</tr>
<tr>
<td>False negatives (patients incorrectly classified as not having HBsAg)</td>
<td>5 (4–6)</td>
<td>18 (15–22)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>True negatives (patients without HBsAg)</td>
<td>12 studies 7552 patients</td>
<td>cohort and case–control type studies</td>
<td>serious</td>
<td>not serious</td>
<td>serious³</td>
</tr>
<tr>
<td>False positives</td>
<td>9 (6–10)</td>
<td>7 (5–9)</td>
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<tr>
<td>Outcome</td>
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<td>Study design</td>
<td>Factors that may decrease quality of evidence</td>
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<tr>
<td>(patients incorrectly classified as having HBsAg)</td>
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1. Lin (7), Lien (8) and Randrianna (9) used a case–control design.
2. Significant heterogeneity across studies for sensitivity; tau-squared 20.2.
3. Heterogeneity exists, but with lower clinical impact; tau-squared 1.56.
References


