Diagnostics for hepatitis B: what we have

Sonjelle Shilton; Deputy Head HCV, Access
World Hepatitis Alliance on-line workshop

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FIND is a global non-profit driving diagnostic innovation to combat major diseases affecting the world’s poorest populations

- WHO Collaborating Centre for Laboratory Strengthening & Diagnostic Technology Evaluation
- WHO SAGE-IVD member
- ISO-certified quality management system for IVD clinical trials

We address market failure by partnering to develop and deliver diagnostic solutions to LMICs

<table>
<thead>
<tr>
<th>ANTIMICROBIAL RESISTANCE</th>
<th>HEPATITIS C &amp; HIV</th>
<th>MALARIA &amp; FEVER</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEGLECTED TROPICAL DISEASES</td>
<td>PANDEMIC PREPAREDNESS</td>
<td>TUBERCULOSIS</td>
</tr>
</tbody>
</table>

Geneva (HQ) South Africa India Viet Nam Kenya
For this webinar, we assume you are already familiar with the basics of HCV diagnostics from a previous webinar and therefore we will not cover IVDs, sensitivity and specificity, or regulatory overviews here.

• You can find this information from min 1:22 to 9:25 in this video here: https://www.worldhepatitisalliance.org/news/nov-2019/catch-find-webinar-hepatitis-c-diagnostics

In this webinar, we will focus on the various tests available for hepatitis B, with a close look at those recommended in the WHO Hepatitis B testing guidelines. We will not cover HBV transmission or disease progression.

• Here is a short overview on viral hepatitis in general: https://www.youtube.com/watch?v=v6XISQ_zQgQ

• And this is a short overview from Western Australia (Department of Health) on hepatitis B: https://www.youtube.com/watch?v=ENlo5JOWL2Q
Global situation

WHO estimates that in 2015, 257 million people were living with chronic hepatitis B infection (defined as hepatitis B surface antigen positive).

In 2015, hepatitis B resulted in an estimated 887,000 deaths, mostly from cirrhosis and hepatocellular carcinoma (i.e. primary liver cancer).

As of 2016, 27 million people (10.5% of all people estimated to be living with hepatitis B) were aware of their infection, while 4.5 million (16.7%) of the people diagnosed were on treatment. According to latest WHO estimates, the proportion of children under five years of age chronically infected with HBV dropped to just under 1% in 2019 down from around 5% in the pre-vaccine era ranging from the 1980s to the early 2000s.

HBV-HIV coinfection: about 1% of people living with HBV infection (2.7 million people) are also infected with HIV. Conversely, the global prevalence of HBV infection in HIV-infected persons is 7.4%. Since 2015, WHO has recommended treatment for everyone diagnosed with HIV infection, regardless of the stage of disease. Tenofovir, which is included in the treatment combinations recommended as first-line therapy for HIV infection, is also active against HBV.
What is the need?

Major gaps in HBV testing: screening and diagnosis — 90% undiagnosed in 2016

Fig. 1. Cascade of care* for hepatitis B treatment, by WHO region, 2016


* The sequential steps or stages of hepatitis B care that persons living with hepatitis B virus infection go through, from diagnosis through viral suppression.
Several different guidelines are currently available


**World Health Organization (WHO)**
- • 2017 Hepatitis B testing guidelines: https://apps.who.int/iris/bitstream/handle/10665/254621/9789241549981-eng.pdf?sequence=1
- • 2020 Hepatitis B prevention of mother to child transmission guidelines: https://apps.who.int/iris/bitstream/handle/10665/333391/9789240002708-eng.pdf?sequence=1&isAllowed=y

**American Association for the Study of Liver Diseases (AASLD)**
- • Guidelines from 2016

**Asian Pacific Association of the Liver (APASL)**

**European Association of the Study of the Liver (EASL)**
Terminology overview
Check out [Hepatitis B Foundation](https://www.hepb.org/prevention-and-diagnosis/diagnosis/other-tests/) for more detailed explanations:

<table>
<thead>
<tr>
<th>Name of test</th>
<th>What is it?</th>
<th>In WHO guideline?</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg (hepatitis B surface antigen)</td>
<td>Tests for a protein found on the surface of the hepatitis B virus</td>
<td>Yes</td>
<td>Part of ‘Hepatitis B Panel’</td>
</tr>
<tr>
<td>Anti-HBs or HBsAb (hepatitis B surface antibody)</td>
<td>Tests for cells your body produces if exposed to hepatitis B virus and/or vaccine</td>
<td>No</td>
<td>Part of ‘Hepatitis B Panel’</td>
</tr>
<tr>
<td>anti-HBc or HBcAb (hepatitis B core antibody)</td>
<td>Tests for cells your body produces if exposed to hepatitis B virus</td>
<td>No</td>
<td>Part of ‘Hepatitis B Panel’</td>
</tr>
<tr>
<td>Anti-HBc IgM or anti-HBc IgG (anti-hepatitis B core IgM or IgG)</td>
<td>Test for cells your body produces if exposed to a hepatitis B virus</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>HBeAg (Hepatitis B e-Antigen)</td>
<td>Test for a part of the hepatitis B virus that the virus usually produces when very active in your body</td>
<td>Yes (PMTCT only)</td>
<td></td>
</tr>
<tr>
<td>anti-HBe or HBeAb (Hepatitis B e-Antibody)</td>
<td>Test for cells your body produces in reaction to the HBeAg</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Hepatitis B Virus DNA Quantification (“viral load”)</td>
<td>Test that can estimate how much hepatitis B virus is in your body</td>
<td>Yes (if available)</td>
<td></td>
</tr>
</tbody>
</table>
WHO HBV testing algorithm
Prevention of mother-to-child transmission of hepatitis B virus: Guidelines on antiviral prophylaxis in pregnancy (July 2020)

Abbreviations:
- ALT: alanine aminotransferase;
- HBV: hepatitis B virus;
- HCC: hepatocellular carcinoma;
- HBeAg: hepatitis B e antigen;
- HBIG: hepatitis B immune globulin;
- HBsAg: hepatitis B surface antigen;
- RDT: rapid diagnostic test

2 At least once and as early as possible in the pregnancy
3 Using clinical criteria and non-invasive tests (APRI score > 2 in adults or Fibroscan)
4 Hepatitis B timely (within 24 hours) birth dose vaccination of the infant followed by 2 or 3 doses of hepatitis B vaccine should be given regardless of HBsAg status of the pregnant mother.
Serological testing for HBV
Algorithm on maternal and infant interventions for prevention of mother-to-child transmission, and assessment of eligibility of mother for treatment for her own health (Based on these guidelines and the 2015 Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection).

**Single assay: laboratory testing (EIA / CIA*) or quality-assured RDT**

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Summary Algorithms

**Fig. 2. Summary algorithm for diagnosis, treatment and monitoring of chronic HBV infection**

*EIA / CIA = enzyme immunoassay / chemiluminescent immunoassay*
Serological tests (also called serological assays), tests that diagnose HBV infection

Serological assays
- Assays that detect the presence of either antigens or antibodies, typically in serum or plasma but also in capillary/venous whole blood and oral fluid. These include rapid diagnostic tests (RDTs) and laboratory-based immunoassays, e.g. enzyme immunoassays (EIAs).

Rapid diagnostic tests (RDTs)
- Immunoassays that detect antigens or antibodies and can give a result in less than 30 minutes.

Enzyme immunoassays (EIAs)
- Immunoassays that detect antigens or antibodies.
Screening for HBV

**Centralized settings**

- **Settings:** well-equipped lab
- **Operator:** qualified lab technician
- **Specimen type:** plasma, serum
- **Turnaround time:** >2 hours

**Decentralized settings**

- **Settings:** primary facility
- **Operator:** trained healthcare worker
- **Specimen type:** capillary blood
- **Turnaround time:** 5-20 min

*Currently off-label; needs field validation

**Dried blood spots**

*CIA chemiluminescent immunoassay
### Hepatitis B rapid diagnostic tests (RDT) that are WHO PQ / ERPD

<table>
<thead>
<tr>
<th>Product name</th>
<th>Manufacturer</th>
<th>Performance</th>
<th>Sample type</th>
<th>WHO PQ?</th>
<th>Stringent Regulatory Authority</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Determine HBsAg 2</td>
<td>Alere Medical Co. Ltd</td>
<td>Sens: 90.8% Spec: 99.1%* From a systematic review so more robust</td>
<td>Serum/plasma/whole blood</td>
<td>Yes</td>
<td>CE mark</td>
</tr>
<tr>
<td>Vikia HBsAg</td>
<td>bioMérieux SA</td>
<td>Sens: 82.5% Spec: 99.9%* From a systematic review so more robust</td>
<td>Serum/plasma/whole blood</td>
<td>Yes</td>
<td>CE mark</td>
</tr>
<tr>
<td>SD Bioline HBsAg WB</td>
<td>Abbott Diagnostics Korea Inc</td>
<td>Sens: 99.46 Spec:99.82%** From 1 study only so not as robust</td>
<td>Serum/plasma/whole blood/ body fluids</td>
<td>Yes</td>
<td>ROW</td>
</tr>
<tr>
<td>First Response® HBsAg Card Test</td>
<td>Premier Medical Corporation</td>
<td>No independent</td>
<td>Serum/plasma/whole blood</td>
<td>No, ERPD</td>
<td>CE mark</td>
</tr>
</tbody>
</table>

1. **WHO PQ / ERPD**: World Health Organization Prequalification / Expert Review Panel for Diagnostics


bioMérieux has informed its customers that this product will be discontinued, effective 1.1.2020.
## Hepatitis B surface antigen EIA, WHO PQ / ERPD

<table>
<thead>
<tr>
<th>Product name</th>
<th>Manufacturer</th>
<th>Sample type</th>
<th>WHO PQ?</th>
<th>Stringent Regulatory Authority</th>
</tr>
</thead>
<tbody>
<tr>
<td>*DS-EIA-HBsAg-0,01</td>
<td>RPC Diagnostics Systems</td>
<td>Serum/plasma</td>
<td>Yes</td>
<td>CE mark</td>
</tr>
<tr>
<td>Murex HBsAg Version 3 with Murex HBsAg Confirmatory Version 3</td>
<td>DiaSorin S.p.A UK Branch</td>
<td>Serum/plasma</td>
<td>Yes</td>
<td>CE mark</td>
</tr>
<tr>
<td>Monolisa HBsAg ULTRA assay</td>
<td>Bio-Rad Laboratories, Marnes La Coquette, France</td>
<td>Serum/plasma</td>
<td>EPRD</td>
<td>CE mark</td>
</tr>
</tbody>
</table>

Combo testing (serology)
6.4.2 Integrating the diagnosis of hepatitis with diagnostic platforms and laboratory services used for other infections

Combination integrated multidisease serological tests

The use of combination integrated blood- or oral-based multidisease assays allow for integrated testing of HIV, HBV and HCV. Using a single specimen improves the efficiency of testing programmes, especially in populations with a high prevalence of HIV/HCV or HBV/HCV coinfection. While not yet fully validated, preliminary results of these combination assays appear promising (160).
## Multiplex serology testing (combo tests)

<table>
<thead>
<tr>
<th>Test</th>
<th>Manufacturer</th>
<th>Detection</th>
<th>Regulatory status (SRA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detect 3 HIV/HCV/HBV combo kit</td>
<td>Artron Laboratories (Canada)</td>
<td>X X X</td>
<td>CE (plasma, serum)</td>
</tr>
<tr>
<td>Triplex HIV, HCV, HBsAg</td>
<td>Biosynex (France)</td>
<td>X X X</td>
<td>NA</td>
</tr>
<tr>
<td>Hep B, Hep C, HIV Combination Rapid Test</td>
<td>Maternova (US)</td>
<td>X X X</td>
<td>NA</td>
</tr>
<tr>
<td>Multiplo HBc/HIV/HCV</td>
<td>MedMira (Canada)</td>
<td>X X X</td>
<td>RUO</td>
</tr>
<tr>
<td>HBsAg/HCV Ab Rapid Test</td>
<td>Spectrum Diagnostics (Egypt)</td>
<td>X X</td>
<td>NA</td>
</tr>
<tr>
<td>Rapid HBsAg/HCV/HIV/Syphilis Combo</td>
<td>Euro Genomas (Lithuania)</td>
<td>X X X</td>
<td>CE</td>
</tr>
<tr>
<td>OnSite HBsAg/HCV Ab Rapid Test</td>
<td>CTK Biotech (US)</td>
<td>X X</td>
<td>NA</td>
</tr>
<tr>
<td>COMBIQUIC HIV/HCV</td>
<td>Qualpro Diagnostics (India)</td>
<td>X X</td>
<td>NA</td>
</tr>
<tr>
<td>TriQuick HIV/HCV/HCV</td>
<td>Genlantis Diagnostics</td>
<td>X X X</td>
<td>NA</td>
</tr>
</tbody>
</table>

Field validation is needed to assess diagnostic accuracy of these tests.
Testing for chronic hepatitis B/PMTCT prophylaxis
2. Prompt or reflex HBV DNA and/or HBeAg testing (PMTCT* only)

*PMTCT = prevention of mother-to-child transmission
**Testing for chronic hepatitis B / PMTCT prophylaxis**

**Centralized settings**
- **Settings:** well-equipped lab
- **Operator:** qualified lab technician
- **Specimen type:** plasma, serum
- **Turnaround time:** >5 hours

**Dried blood spots**
- Currently off-label; needs field validation

**Decentralized settings**
- **Settings:** district hospitals
- **Operator:** trained healthcare worker
- **Specimen type:** capillary blood/plasma
- **Turnaround time:** 60-90 min
# HBV virological technologies that have completed / ERPD

<table>
<thead>
<tr>
<th>Product name</th>
<th>Manufacturer</th>
<th>Sample type</th>
<th>WHO PQ?</th>
<th>Stringent Regulatory Authority</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alinity m HBV</td>
<td>Abbott Molecular</td>
<td>Serum/Plasma</td>
<td>ERPD</td>
<td>CE mark</td>
</tr>
<tr>
<td>COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, version 2.0</td>
<td>Roche</td>
<td>Serum/Plasma</td>
<td>ERPD</td>
<td>CE mark</td>
</tr>
<tr>
<td>artus HBV RG RT-PCR Kit (AS - Rotor-Gene Q)</td>
<td>QIAGEN GmbH,</td>
<td>Serum/Plasma</td>
<td>ERPD</td>
<td>CE mark</td>
</tr>
<tr>
<td>artus HBV QS-RGQ Kit (QIAsymphony® DSP / AS - Rotor-Gene Q)</td>
<td>QIAGEN GmbH,</td>
<td>Serum/Plasma</td>
<td>ERPD</td>
<td>CE mark</td>
</tr>
</tbody>
</table>
Each molecular instrument possesses unique features, which need to be considered when defining the optimal device mix within a lab network

<table>
<thead>
<tr>
<th>Instrument / Supplier</th>
<th>Cobas 4800-6800-8800 / Roche</th>
<th>CAP/CTM 96 / Roche</th>
<th>M2000sp / Abbott</th>
<th>Panther / Hologic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assays</strong></td>
<td>HIV (EID;VL);HPV;HCV;HBV;TB</td>
<td>HIV (EID;VL);HCV;HBV;TB</td>
<td>HIV (EID;VL);TB;HPV;HCV;HBV</td>
<td>HIV (EID;VL);HPV;HCV;HBV</td>
</tr>
<tr>
<td><strong>HIV sample types</strong></td>
<td>DBS; Plasma; PSC (VL)</td>
<td>DBS; Plasma</td>
<td>DBS; Plasma</td>
<td>DBS (VL); Plasma</td>
</tr>
<tr>
<td><strong>HBV sample types</strong></td>
<td>Plasma</td>
<td>Plasma</td>
<td>Serum; Plasma</td>
<td>Serum; Plasma</td>
</tr>
<tr>
<td><strong>HCV sample types</strong></td>
<td>Plasma (PSC and DBS in the pipeline)</td>
<td>Plasma</td>
<td>DBS; Serum; Plasma</td>
<td>Serum; Plasma (DBS in the pipeline)</td>
</tr>
</tbody>
</table>

**Infrastructure requirements**

<table>
<thead>
<tr>
<th>Space requirements</th>
<th>1.8 m$^2$ - 5.5 m$^2$, Fixed</th>
<th>3 m$^2$, Fixed</th>
<th>3.15. m$^2$, Fixed</th>
<th>1 m$^2$, Moveable</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human resources</strong></td>
<td>1-2 FTEs/machine</td>
<td>1-2 FTEs/machine</td>
<td>1-2 FTEs/machine</td>
<td>1 FTE/&lt;4 machines</td>
</tr>
</tbody>
</table>

**Workflow requirements**

<table>
<thead>
<tr>
<th>Workflow</th>
<th>Batch</th>
<th>Batch</th>
<th>Batch</th>
<th>Random access</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throughput (8hr)</td>
<td>192-960</td>
<td>168</td>
<td>96</td>
<td>320</td>
</tr>
<tr>
<td>Time to first result (hr)</td>
<td>&lt; 3.5</td>
<td>~5.5</td>
<td>~5.5</td>
<td>&lt; 3.5</td>
</tr>
</tbody>
</table>
Point of care (POC) and near POC
Near-POC HBV DNA assays available on the market

<table>
<thead>
<tr>
<th>PLATFORM</th>
<th>Xpert HBV VL assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAMPLE TYPE</td>
<td>Plasma</td>
</tr>
<tr>
<td>SENSITIVITY</td>
<td>99%</td>
</tr>
<tr>
<td>SPECIFICITY</td>
<td>100%</td>
</tr>
<tr>
<td>SAMPLE PREPARATION</td>
<td>Integrated</td>
</tr>
<tr>
<td>TIME TO RESULT</td>
<td>110 min</td>
</tr>
<tr>
<td>REGULATORY STATUS</td>
<td>CE-IVD</td>
</tr>
<tr>
<td>POWER SUPPLY</td>
<td>Need electricity supply</td>
</tr>
<tr>
<td>DATA ANALYSIS</td>
<td>PC</td>
</tr>
<tr>
<td>TEST MENU</td>
<td>TB, HIV, HCV and many others</td>
</tr>
<tr>
<td>TEST COST</td>
<td>US$ 17+ ex works</td>
</tr>
<tr>
<td>INSTRUMENT COST</td>
<td>US$ 17,500</td>
</tr>
</tbody>
</table>
HBeAg RDTs
**HBVeAg performance: very limited information available**


### Table 3

Performance of RDTs for HBeAg detection (Group 1, Group 2 and East Asian samples)

<table>
<thead>
<tr>
<th></th>
<th>Senegalese samples</th>
<th>East Asian samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1: HBeAg-positive cases (N = 48)</td>
<td>Group 2: HBeAg-negative controls (N = 196)</td>
</tr>
<tr>
<td></td>
<td>Sensitivity 95% CI</td>
<td>Specificity 95% CI</td>
</tr>
<tr>
<td>SD Bioline</td>
<td>29.8% (14/47) 17.3–44.9</td>
<td>100% (196/196) 98.1–100</td>
</tr>
<tr>
<td>Insight</td>
<td>31.1% (14/45) 18.2–46.6</td>
<td>100% (196/196) 98.1–100</td>
</tr>
<tr>
<td>OneStep</td>
<td>42.5% (17/40) 27.0–59.1</td>
<td>98.4% (183/186) 95.4–99.7</td>
</tr>
</tbody>
</table>

HBeAg = hepatitis B e antigen; RDT = rapid diagnostic test.

*Discordant results on each RDT between two laboratory staff were excluded (SD Bioline, N = 1; Insight, N = 3; OneStep, N = 2).
Review commissioned by WHO for PMTCT Guidelines

Note, this systematic review only included 1 study that used an HBeAg RDT (SD Bioline in Cambodia, which included 128 people who were reference tested) out of the 82 studies included in the systematic review. No HIV+ included in this study.

There is a paucity of evidence on the performance of HBeAg RDTs; more evidence is needed, especially among HIV+ women, if moving to dolutegravir (DTG) regimens from tenofovir disoproxil fumarate/tenofovir alafenamide (TDF/TAF).

<table>
<thead>
<tr>
<th>Sensitivity (95% CI range)</th>
<th>Specificity (95% CI range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥5 log IU.ml</td>
<td>76.5</td>
</tr>
<tr>
<td>≥6 log IU.ml</td>
<td>78.8</td>
</tr>
<tr>
<td>≥7 log IU.ml</td>
<td>89.3</td>
</tr>
</tbody>
</table>

Liver staging
Assess stage liver disease using NITs* (APRI, FIB-4, TE)

*NITs = non-invasive tests
(APRI, aminotransferase/platelet ratio index; FIB4, fibrosis-4 score; TE, transient elastography)
## Existing liver staging options

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
<th>Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochem</td>
<td>Uses blood test for a blood test to measure your aspartate aminotransferase (AST) and a platelet count</td>
<td>Machines needed to conduct the blood tests can usually be found at level 1 health centers. Is relatively inexpensive</td>
</tr>
<tr>
<td>FIB4</td>
<td>Is a formula based on several laboratory tests: (Age x AST) / (Plts x (sqr(ALT)))</td>
<td></td>
</tr>
<tr>
<td>Fibroscan</td>
<td>Machine which can provide liver staging results</td>
<td>Machine is expensive</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>Can be used to provide liver staging results</td>
<td>Requires trained technician</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Is not widely available in all countries/contexts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>While often machines are already in place for other services requires trained technician</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wait times for ultrasound appointment can be long as other patient types may be prioritized (pregnant women)</td>
</tr>
</tbody>
</table>
We believe
Simple, rapid, robust and affordable diagnostic solutions bring game-changing possibilities above and beyond their immediate benefit.

We believe
Our work can spark real progress in the health of lower- and middle-income countries and their populations.

We believe
With improved health comes greater hope: individuals empowered to support their families, revive businesses, and thrive in school.

Thank you!