Point Of Care (POC) Testing
An unmet and urgent need

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Hepatitis B Virus Serum Biomarkers Virtual Workshop October 12, 2020
Worldwide Deaths From Chronic Viral Hepatitis

No. of Deaths (millions)


- Chronic HBV and HCV infection
- Tuberculosis
- HIV infection
- Malaria

D. Thomas, N Engl J Med 2019
Similarities between HBV and HCV
- Blood safety
- Injection safety
- Harm reduction

Major difference between HBV and HCV
- Effective HBV vaccine is available
- HBV testing for pregnant women
- For HBsAg (+) women
  - HBlg for new born in addition to vaccine
  - Antiviral therapy during 3rd trimester for women with HBV DNA >200,000 IU/ml
Causes of Death from Viral Hepatitis

Number of deaths

- Hepatitis A (HAV)
- Hepatitis B (HBV)
- Hepatitis C (HCV)
- Hepatitis E (HEV)

Number of deaths:

- Hepatocellular carcinoma
- Cirrhosis
- Acute hepatitis

No. of Deaths

- 2015: 884,000
- 2020: 309,000
- 2030: 309,000

HBV
HCV

10% reduction by 2020
65% reduction by 2030
Urgent Need for An Accelerated Rate of Diagnosis and Treatment

HBV

<table>
<thead>
<tr>
<th></th>
<th>2015</th>
<th>2030 Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>90%</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>&lt;9%</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suppression</td>
<td></td>
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</tr>
</tbody>
</table>
HBsAg screening test:
- Rapid diagnostic test (RDT)
- Lab-based immunoassay (ELISA)

Assessment for treatment:
- HBV DNA Nucleic Acid Test (NAT)
- Fibrosis measurement
  - Transient Elastography (Fibroscan)
  - *APRI score *(AST/ULN AST)/Platelet
- ALT (liver panel)

Monitoring:
- HCC surveillance: ultrasound +/- AFP
- ALT (liver panel)
- HBV DNA
- HBsAg (Lab based)
- Renal function
POC Approach Needs to Meet the ASSURED Criteria

- RDTs
- Dried Blood Spots (DBS)

A = Affordable
S = Sensitive
S = Specific
U = User-friendly (simple)
R = Robust and rapid
E = Equipment-free or minimal
D = Deliverable to those who need them

Modified from FIND presentation
HBsAg screening test:
- Rapid diagnostic test (RDT) Available
- Lab-based immunoassay

Assessment for treatment:
- HBV DNA Nucleic Acid Test (NAT) Promising platforms in development, DBS
- Fibrosis measurement
  - Portable Fibroscan Available
  - *APRI score
- ALT

Monitoring:
- HCC surveillance:
  - Portable Ultrasound Available
  - +/- AFP
- ALT (liver panel)
- HBV DNA
- HBsAg (Lab-based immunoassay)
- Renal function
The test strip consists:
1) HBsAb conjugates on pad
2) Nitrocellulose membrane strip
   - Test band
   - Control band

1. NEGATIVE RESULT: If only the C band is developed, the test indicates that the level of HBsAg in the specimen is undetectable

2. POSITIVE RESULT: If both C and T bands are developed, the test indicates that the specimen contains HBsAg
Performance Evaluation of 70 HBsAg Assays on Samples with Various HBV Genotypes and HBsAg Subtypes

HBsAg ELISA Tests

Rapid Tests
## Specifications of Available HBsAg RDTs

<table>
<thead>
<tr>
<th>Test</th>
<th>Manufacturer</th>
<th>Nature of device</th>
<th>Matrices</th>
<th>Volume needed</th>
<th>Time to result</th>
<th>CE-marked</th>
<th>FDA-approved</th>
<th>WHO-prequalified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determine™ HBsAg</td>
<td>Alere, Waltham, MA</td>
<td>Lateral flow</td>
<td>Whole blood, serum, plasma</td>
<td>50 µl</td>
<td>15 min</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>VIKIA® HBsAg</td>
<td>bioMérieux, Marcy-l’Étoile, France</td>
<td>Lateral flow</td>
<td>Whole blood, serum, plasma</td>
<td>75 µl</td>
<td>15–30 min</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>DRW HBsAg rapid Test</td>
<td>Diagnostics for the Real World, San Jose, CA</td>
<td>Lateral flow</td>
<td>Serum, plasma</td>
<td>80 µl</td>
<td>30 min</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Toyo HBsAg Rapid Test</td>
<td>TurkLab, Izmir, Turkey</td>
<td>Flow-through</td>
<td>Whole blood, serum, plasma</td>
<td>100 µl</td>
<td>5–15 min</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Assure HBsAg Rapid Test</td>
<td>MP Biomedicals, Singapore</td>
<td>Flow-through</td>
<td>Whole blood, serum, plasma</td>
<td>50 µl (whole blood) or 75 µl (serum, plasma)</td>
<td>15–20 min</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>First Response HBsAg Card Test</td>
<td>Premier Medical Corporation, Daman, India</td>
<td>Flow-through</td>
<td>Serum, plasma</td>
<td>50 or 75 µl</td>
<td>5–10 min</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>SD Bioline HBsAg</td>
<td>Standard Diagnostics, Yongin, Korea</td>
<td>Flow-through</td>
<td>Whole blood, serum, plasma</td>
<td>100 µl</td>
<td>20 min</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

CE, European Conformity; FDA, US Food and Drug Administration; HBsAg, hepatitis B surface antigen; WHO, World Health Organization.

S.Chevaliez, JM PAWLOTSKY. J Hepatology 2018
## Performance of Determine and Vikia HBsAg RDTs

<table>
<thead>
<tr>
<th>Determine</th>
<th>Year</th>
<th>Country</th>
<th>HIV Status</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>Daviess et al</td>
<td>Malawi</td>
<td>Rx naïve HIV(+)</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>2010</td>
<td>Geretti et al</td>
<td>Ghana</td>
<td>HIV(+)</td>
<td>69.3%</td>
<td>100%</td>
</tr>
<tr>
<td>2012</td>
<td>Hoofman et al</td>
<td>South Africa</td>
<td>Rx naïve HIV(+)</td>
<td>75%</td>
<td>99.6%</td>
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<tr>
<td>2013</td>
<td>Bottero et al</td>
<td>France</td>
<td>Community</td>
<td>93.6%</td>
<td>100%</td>
</tr>
<tr>
<td>2013</td>
<td>Franzeck et al</td>
<td>Tanzania</td>
<td>Rx naïve HIV(+)</td>
<td>96%</td>
<td>100%</td>
</tr>
<tr>
<td>2015</td>
<td>Njai HF et al</td>
<td>Gambia</td>
<td>Community</td>
<td>88.5%</td>
<td>100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vikia</th>
<th>Year</th>
<th>Country</th>
<th>HIV Status</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>Geretti et al</td>
<td>Ghana</td>
<td>HIV(+)</td>
<td>70.7%</td>
<td>100%</td>
</tr>
<tr>
<td>2013</td>
<td>Bottero et al</td>
<td>France</td>
<td>Community</td>
<td>96.5%</td>
<td>99.9%</td>
</tr>
<tr>
<td>2015</td>
<td>Njai HF et al</td>
<td>Gambia</td>
<td>Community</td>
<td>90%</td>
<td>99.8%</td>
</tr>
</tbody>
</table>
Sensitivity of HBsAg ELISA and RDTs can be variable in different HBV genotypes

Genetic variability in the S gene additionally affects diagnostic efficacy and specificity

HBsAg tests that include multiple monoclonal antibodies in the capture phase together with a polyclonal conjugate phase are more accurate

RDTs generally are less sensitive than lab-based ELISA tests

HIV HBV coinfected patients: low HBsAg level in HIV Rx pts

HBsAg RDTs can be applied as a screening test but not ideal for monitoring treatment response
Laboratory for In-House RT-PCR Assay

In-house qPCR test: 8 euros/test

INSERM Lyon/MRC the Gambia

Slide courtesy of Mark Thursz
Advantages of DBS:

- Capillary finger-stick, does not require trained health worker
- Low blood volume
- Does not require basic lab facilities or electric power
- Does not require cold chain for transport, storage

WHO 2017 conditionally recommended use of DBS specimens as an option for HBV DNA NAT in settings:

- No facilities or expertise to take venous blood specimens
- For persons with poor venous access
HBV DNA detection: DBS vs. Serum Samples

Meta-analysis of 12 studies: 5 Europe, 4 Africa, 2 India, 1 Mexico

- DBS sensitivity: 93% to 100%
- DBS specificity: 70% to 100%

HBV DNA detection limits from these DBS studies ranged from 900 IU/ml to 4,000 IU/ml

Potential issues:
- Variable length of storage before testing
- Variable temperatures
- No manufacturer validation for the use of their assays with DBS samples or standardization of technical guidance
Xpert® HCV Viral Load FS assay (Cepheid, CA, USA)
- Fully automated qRT-PCR assay.
- Combines sample preparation, nucleic acid extraction, amplification and detection of target sequences in one cartridge (1 kg).
- LoD: 40 IU/ml
- Uses 100 ul capillary whole blood. Result in 60 min.
- CE-marked, WHO prequalified

Genedrive ® (Manchester, UK)
- Semiquantification of HCV RNA from 30 ul of plasma in <90 Min
- 99.8% sensitivity with HCV RNA >1000 IU/ml
- WHO approved in LMIC

Alere™q (Abbott, Chicago, IL)
- Fully automated, cartridge-based assay
- CE-marked for rapid HIV RNA detection
Sensing HBV in a Golden Sandwich

Amplify the nanoplasmonnic signal by a nanoparticle sandwiching strategy

HBV capture

First Ab-AuNP In 96-well plate

Sandwiching with Second Ab-AuNP

Cirrhosis Dx: Fibroscan Vs. APRI/ FIB4 in HBV

Vibration-controlled transient elastography (Fibroscan)

Cut-off: 13.8 kPa
Sensitivity 93.1%
Specificity 91.1%
PPV 67.5%
NPV 98.5%

Limitations:
Inflammation or non-fasting state can falsely increase scores.

Zhu X Dig Dis Sci (2011)
To reach the WHO goals of HBV elimination:

- Establish the POC model in resource-limited settings.
- Screening: HBsAg RDTS need standardization
- Treatment decision: Ideally, HBV DNA RDT, ALT RDT, or Standardized DBS – HBV DNA, and possibly ALT
- Fibrosis Assessment: Portable transient elastography preferred
- HCC surveillance: Ultrasound, need reliable HCC biomarkers
- Therapy response assessment: low LoD of HBV DNA, qHBsAg
ICE HBV : POC Model Working Team

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