Screening and Diagnosis of Hepatitis Virus Infections

Prof. Jean-Michel Pawlotsky, MD, PhD

National Reference Center for Viral Hepatitis B, C and delta
Department of Virology & INSERM U955

Henri Mondor Hospital
University of Paris-Est
Créteil, France
I

Why Should we Screen for HBV and HCV?
Worldwide Mortality from HCC (Global Disease Burden)

(Cowie et al., AASLD 2013)
Worldwide Mortality from Liver Disease (Global Disease Burden)

- HBV cancer: 400,000 deaths/year
- HBV cirrhosis: 300,000 deaths/year
- HCV cirrhosis: 200,000 deaths/year
- Alcohol cirrhosis: 100,000 deaths/year
- HCV cancer: 0 deaths/year
- Alcohol cancer: 0 deaths/year

(Cowie et al., AASLD 2013)
Importance of Screening

• Worldwide HBV and HCV prevalence is high (240 and 184 million, respectively)

• Chronic HBV and HCV infections are associated with silent disease until serious complications occur

• New therapies are available with very high virological success rates
Histology Results Over 5 Years of Tenofovir (HBeAg+ and -)

Knodell necroinflammatory score

Ishak fibrosis score

(Marcellin et al., Lancet 2013;381:468-75)
HCC incidence was reduced on TDF compared to the natural history model.

In non-cirrhotics, the effect was visible after 2-3 years, significant after 6 years.

(Kim et al., EASL 2013)
Sofosbuvir/Ledipasvir FDC ± RBV

ION-1-Phase III, Gen 1, Rx-naive, 16% cirrhosis

(SOF/LDV) 99%  (SOF/LDV+RBV) 97%  (SOF/LDV) 98%  (SOF/LDV+RBV) 99%
N=214  N=217  N=217  N=217

12 weeks  24 weeks

(Mangia et al., EASL 2014)
II

Diagnosis of HBV and HCV Infections
Diagnosis of Chronic HBV Infection

- HBsAg by ELISA

- HBV DNA by real-time PCR

- HBeAg/Anti-HBe antibodies by ELISA
  - HBeAg-positive hepatitis B
  - HBeAg-negative hepatitis B
Diagnosis of Chronic HCV Infection

- Anti-HCV antibodies by ELISA
- HCV RNA by real-time PCR
III

Alternative Tests for Screening and Diagnosis
Rationale

- Improve large-scale screening in high- and middle-risk populations
- Promote access to care and therapy
- Reduce liver disease progression
- Prevent transmission
- Promote vaccination (HBV)
Alternative Tests

- Point-of-care tests (POCT)
  - Rapid diagnostic tests (immunology-based)
  - Molecular biology tests

- Dried blood spots
Rapid Diagnostic Tests (RDTs)
Interest of RDTs

• They can be used at the patient care’s site
  - Physicians’ offices (GP)
  - Emergency room, ICU
  - Outpatient clinics, rural area, even patients’ homes

• They use original specimen matrix
  - Oral fluid
  - Fingerstick whole blood
  - Venipuncture whole blood
  - Serum or plasma
# Antibody Concentrations in Different Matrices

<table>
<thead>
<tr>
<th>Specimen</th>
<th>IgG (mg/L)</th>
<th>IgM (mg/L)</th>
<th>IgA (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>14730</td>
<td>1280</td>
<td>2860</td>
</tr>
<tr>
<td>Parotid saliva</td>
<td>0.36</td>
<td>0.43</td>
<td>39.5</td>
</tr>
<tr>
<td>Crevicular fluid</td>
<td>3500</td>
<td>250</td>
<td>1110</td>
</tr>
<tr>
<td>Whole saliva</td>
<td>14.4</td>
<td>2.1</td>
<td>19.4</td>
</tr>
</tbody>
</table>
Principle of an RDT

Example of the Oraquick Test

Flat pad

Test line

Control line

Immunochromatography

Cellulose membrane

Human anti-antibody antibodies

HCV antigens (core, NS3, NS4)

Colloidal gold particles bound to protein A
## HBsAg RDTs

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>VIKIA® HBsAg</th>
<th>DRW-HBsAg</th>
<th>Toyo® HBsAg</th>
<th>Determine™ HBsAg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specimen types</strong></td>
<td>whole blood, serum, plasma</td>
<td>serum, plasma</td>
<td>whole blood, serum, plasma</td>
<td>whole blood, serum, plasma</td>
</tr>
<tr>
<td><strong>Volume required (µL)</strong></td>
<td>75</td>
<td>80</td>
<td></td>
<td>50</td>
</tr>
<tr>
<td><strong>Time to result (min)</strong></td>
<td>15-30</td>
<td>30</td>
<td>5-15</td>
<td>15</td>
</tr>
</tbody>
</table>
Performance of HBsAg RDTs from Whole Blood

- 2472 to 3928 individuals were tested (according to the test)
- Results were interpretable in the majority of cases (Determine™: 100%; Vikia®: 99.8%; Quick Profile™: 98.1%)

<table>
<thead>
<tr>
<th></th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determine™</td>
<td>100%</td>
<td>93.6%</td>
<td>100%</td>
<td>99.9%</td>
</tr>
<tr>
<td>VIKIA®</td>
<td>99.9%</td>
<td>96.5%</td>
<td>97.6%</td>
<td>99.9%</td>
</tr>
<tr>
<td>Quick Profile™</td>
<td>99.7%</td>
<td>90.5%</td>
<td>88.4%</td>
<td>99.8%</td>
</tr>
</tbody>
</table>

(Bottero et al., J Hepatol 2013;58:473-8)
Performance of HBsAg Detection in HIV+ Patients in Tanzania

272 treatment-naïve HIV-positive subjects (9.2% HBsAg+)

<table>
<thead>
<tr>
<th>Test</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determine™ HBsAg</td>
<td>100%</td>
<td>96.0%</td>
<td>100%</td>
<td>99.5%</td>
</tr>
</tbody>
</table>

(Franzeck et al., PlosOne 2013;8:e58468)
# HCV Antibody RDTs

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Oraquick® HCV</th>
<th>Toyo® HCV</th>
<th>Labmen® HCV</th>
<th>Signal HCV v2.0</th>
<th>HCV TriDot 4&lt;sup&gt;th&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specimen type</strong></td>
<td>Orasure</td>
<td>Turklab</td>
<td>Turklab</td>
<td>Span Diagnostics</td>
<td>J Mitra &amp; Co</td>
</tr>
<tr>
<td>oral fluid, whole</td>
<td></td>
<td>whole blood, serum, plasma</td>
<td>whole blood, serum, plasma</td>
<td>serum, plasma</td>
<td>serum, plasma</td>
</tr>
<tr>
<td>blood, serum, plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Volume required (µL)</strong></td>
<td>40 (oral fluid)</td>
<td>30</td>
<td>10</td>
<td>100</td>
<td>One drop</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Time to read (min)</strong></td>
<td>20-40</td>
<td>15</td>
<td>15</td>
<td>10</td>
<td>3</td>
</tr>
</tbody>
</table>
Performance of HCV Antibody RDTs

*Fingerstick whole blood*

<table>
<thead>
<tr>
<th>Test</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>OraQuick® HCV Rapid Ab Test</td>
<td>100%</td>
<td>99.4%</td>
<td>100%</td>
<td>98.4%</td>
</tr>
<tr>
<td>TOYO® anti-HCV test</td>
<td>98.2%</td>
<td>96.2%</td>
<td>99.0%</td>
<td>93.1%</td>
</tr>
<tr>
<td>Labmen® HCV test</td>
<td>100%</td>
<td>62.7%</td>
<td>100%</td>
<td>49.6%</td>
</tr>
</tbody>
</table>

318 HCV-positive, 171 HCV-negative

(Chevaliez et al., manuscript in preparation)
## Performance of HCV Antibody RDTs
### Crevicular fluid (Oraquick test)

318 HCV-positive, 171 HCV-negative

<table>
<thead>
<tr>
<th>Test</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>OraQuick® HCV Rapid Ab Test</td>
<td>100%</td>
<td>98.2%</td>
<td>100%</td>
<td>96.6%</td>
</tr>
</tbody>
</table>

(Chevaliez et al., manuscript in preparation)
Performance of HCV Antibody RDTs

*Crevicular fluid (Oraquick test)*

(Chevaliez et al., manuscript in preparation)
Performance of HCV Antibody RDTs in High-Risk Populations

Crevicular fluid (Oraquick test)

- 503 IVDUs and immigrants from HCV-endemic countries in NY, April-September 2009
- Valid results in 96.6%
- OraQuick® result concordant with EIA in 97.5% (474/486)
- Discrepancies in 12 participants (2.5%)
  - HCV RNA detection performed in 10 subjects
  - In 6/10 tested, OraQuick in agreement with HCV RNA

(Drobnik et al., Am J Public Health 2011;101:2151-5)
Non-immunological POCTs
(Viral genome detection/quantification)
Alere™ Q for Viral Genome Detection
Alere™ Q for Viral Genome Detection
Technology Pipeline for Viral Load and EID

2012

2013

2014

2015

2016

Liat

Alere Q

WAVE 80 EOSCAPE

Lynx EID

SAMBA VL

SAMBA EID

Cavidi AMP

Micronics

ALL

Biohelix

NWGHF VL

Gene Xpert

Lumora

*Estimated - timeline and sequence may change
Dried blood spots
Value of DBS

• Advantages
  - Low volume required (50-70 µL)
  - Good stability of the biological matrix
  - Easy to collect and mail at room temperature
  - Serological, molecular and pharmacological analysis are available

• Disadvantages
  - Lower analytical sensitivity than the classical biological matrix (serum, plasma)
  - No standardized procedures
  - Storage at -20°C may be required
HBsAg and HBV DNA in DBS

LLOD = 0.30 ± 0.08 IU/mL
N = 100 HBsAg-positive patients

LLOD = 914 ± 158 IU/mL
N = 50 HBV DNA-positive patients

(Mohamed et al., PlosOne 2013;8:e61077)
HCV Markers in DBS

Anti-HCV antibodies (3rd-gen EIA)

340 HCV-positive
171 HCV-negative

(Chevaliez et al., manuscript in preparation)
HCV Markers in DBS

HCV core antigen

(Chevaliez et al., manuscript in preparation)
HCV Markers in DBS

HCV RNA (m2000, Abbott)

(Chevaliez et al., manuscript in preparation)

![Graph showing HCV RNA levels in whole blood from DBS for different genotypes.](attachment:image.png)

HCV RNA levels in whole blood from DBS (Log IU/mL)

- Genotype 3 (n=37)
- Genotype 2 (n=17)
- Genotype 1 (n=178)
- Genotype 4 (n=55)
- Genotype 6 (n=4)
- Genotype 5 (n=3)

$r=0.87; p<0.001$
HCV Markers in DBS

HCV RNA (CAP/CTM, Roche)

Chevaliez et al., manuscript in preparation

HCV RNA levels in whole blood from DBS (Log IU/mL)

Genotype 3 (n=37)
Genotype 2 (n=17)
Genotype 1 (n=178)
Genotype 4 (n=55)
Genotype 6 (n=4)
Genotype 5 (n=3)

$r=0.89; p<0.001$

(Chevaliez et al., manuscript in preparation)
HCV Markers in DBS

**HCV RNA**

- Mean differences in HCV RNA levels between serum and whole blood from DBS
  - $1.76 \pm 0.33 \text{ Log IU/mL with } m2000$
  - $1.57 \pm 0.30 \text{ Log IU/mL with CAP/CTM v2.0}$

(Chevaliez et al., manuscript in preparation)
HCV Markers in DBS

\( HCV \) genotype

- HCV genotype determination from DBS (N=263)
  - 100% concordance at the genotype level
  - Subtyping failed in 16 cases and was erroneous in one case

(Chevaliez et al., manuscript in preparation)
Conclusions

• Active screening for HBV and HCV is mandated because
  • These infections are highly prevalent
  • They are silent until late stages of liver disease
  • They are associated with higher mortality rates
  • Efficient treatments are available

• Rapid diagnostic tests and DBS offer opportunities for early screening, diagnosis and access to care