“The Alphabet Soup of Viral Hepatitis Testing”

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Objectives

• Understand the appropriate use of laboratory tests for diagnosing and monitoring HBV infection

• Understand the laboratory testing algorithm for HCV infection
Hepatitis A Virus (HAV)

- Picornavirus
- ssRNA, ~7500 bp
- Enteric transmission
- 15-45 day incubation, mild disease
- No chronic infection state, but can kill
Tests for HAV

• Anti-HAV
  ➢ total antibody (IgG and IgM)
  ➢ indicates present or past infection, or response to vaccination
  ➢ positive in 40-50% of tested patients
  ➢ should be ordered to assess immunity

• Anti-HAV IgM
  ➢ detected for 3-6 months
  ➢ indicates current or recent infection
  ➢ positive in 0.40 % of patients tested
  ➢ should be ordered if patient meets CDC HAV clinical criteria to diagnose acute hepatitis A
Hepatitis B Virus (HBV)

“serum” hepatitis

- Hepadnavirus
- Partially dsDNA, ~3200 bp
- 50-150 day incubation
- 10% become chronically infected if exposed as an adult
- 90% become chronically infected if exposed as an infant (perinatal transmission)

CDC. Surveillance for Acute Viral Hepatitis- United States, 2007. MMWR.2009
Recent Hepatitis B Guidelines

Recommendations for Identification and Public Health Management of Persons with Chronic Hepatitis B Virus Infection

Prepared by
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National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Division of Viral Hepatitis

AASLD PRACTICE GUIDELINES

Chronic Hepatitis B
Anna S. F. Lok1 and Brian J. McMahon2

NIH CONFERENCE | Annals of Internal Medicine

National Institutes of Health Consensus Development Conference Statement: Management of Hepatitis B
Michael F. Sorrell, MD; Edward A. Belongia, MD; Jose Costa, MD; Ilana F. Gareen, PhD; Jean L. Grem, MD; John M. Inadomi, MD; Earl R. Kern, PhD; James A. McHugh, MD; Gloria M. Petersen, PhD; Michael F. Rein, MD; Doris B. Strader, MD; and Hartwell T. Trotter, MS
# New Recommendations for Routine Testing for Chronic HBV

<table>
<thead>
<tr>
<th>Population</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persons born in regions of high and intermediate HBV prevalence (&gt;2%)</td>
<td>New (for intermediate prevalence of &gt;2%)</td>
</tr>
<tr>
<td>Injection-drug abusers</td>
<td>New</td>
</tr>
<tr>
<td>MSM</td>
<td>New</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>CDC 2001</td>
</tr>
<tr>
<td>All pregnant women</td>
<td>CDC 2005</td>
</tr>
<tr>
<td>Infants born to HBsAg-positive mothers</td>
<td>CDC 2005, 2007, 2008</td>
</tr>
<tr>
<td>Donors of blood, plasma, organs, tissue and semen</td>
<td>Code of Federal Regulations (FDA)</td>
</tr>
<tr>
<td>US born persons not vaccinated as infants whose parents were born in region of high HBV prevalence (&gt;8%)</td>
<td>New</td>
</tr>
<tr>
<td>Persons with elevated ALT/AST of unknown etiology</td>
<td>New</td>
</tr>
<tr>
<td>HIV-positive persons</td>
<td>CDC 2004</td>
</tr>
<tr>
<td>Household, needle-sharing or sex contacts of persons known to be HBsAg positive</td>
<td>CDC 2005</td>
</tr>
<tr>
<td>Persons who are sources for exposures (needle-stick, sexual assault)</td>
<td>CDC 2004</td>
</tr>
<tr>
<td>Persons needing immunosuppressive therapy (transplant, rheumatology and gastroenterology)</td>
<td>New</td>
</tr>
</tbody>
</table>
Serological Tests For HBV

Antibody detection

- Anti-HBs
- Anti-HBe
- Anti-HBc (IgM, total)

Antigen detection

- HBeAg
- HBsAg
Acute HBV Infection

Typical response when patient recovers

- **HBsAg**: Hepatitis B surface antigen
- **HBeAg**: Hepatitis B e antigen
- **HBV DNA**: Hepatitis B virus DNA
- **Symptoms**: Clinical symptoms
- **ALT**: Alanine transaminase
- **IgM Anti-HBc**: IgM antibody to hepatitis B core antigen
- **Anti-HBs**: Antibody to hepatitis B surface antigen
- **Anti-HBc**: Antibody to hepatitis B core antigen
- **Anti-HBe**: Antibody to hepatitis B e antigen

Months following Infection

Years
Chronic HBV Infection

Typical response with mild disease

Months following Infection

Symptoms
ALT
HBsAg
HBeAg
HBV DNA
IgM
Anti-HBc
Anti-HBe
Chronic HBV Infection

Typical response with severe disease

- Symptons
- ALT
- HBsAg
- HBeAg
- HBV DNA
- Anti-HBc
- IgM
- Anti-HBe

Months following Infection

0 1 2 3 4 5 6 7 8 9

years
Hepatitis B Virus (HBV)

Structure of the Virus and other particles

- Infective virus (Dane particle)
- Non-infective sphere
- Surface Antigen
- Nucleocapsid
- Non-infective tubule
HBV Surface Antigen (HBsAg)

- 10,000 HBsAg to 1 infectious virion
- Qualitative assay
  - most require confirmation of all positives
  - some require confirmation only of results in low positive range
  - confirmation is achieved by neutralization
Hepatitis B Surface Antigen Testing (HBsAg)

**Initial Test**
- Reactive > Threshold level (in the “Hot Zone”)
  - Report Positive
- Reactive < Threshold level
  - Repeat in Duplicate
  - 1 or 2 Reactive → Go to Confirmatory Assay
  - 2/2 Non-Reactive → Report Negative
- Non-Reactive → Report Negative
HBsAg Confirmatory Assay

Patient sample is incubated with blocking antibody or diluent. HBsAg assay is performed and if there is > 50% reduction in signal between the two aliquots (A & B) then it is interpreted as >50% neutralization and therefore confirmed for HBV surface antigen.
HBV Surface Antigen Antibody (anti-HBs)

• Detects the protective HBV antibody

• FDA approved qualitative and quantitative assays

• Protection: 10 IU/L of active antibody or 100-150 IU/L of passive antibody

• Cannot distinguish actively acquired antibody from passively acquired
HBV Core Total IgM (anti-HBc IgM)

- Most consistent test for acute infection
- May be weakly positive in ~10% of chronic HBV cases (likely reactivation)
- False positives can occur
HBV e Antigen and Antibody (HBeAg/anti-HBe)

- Useful in monitoring chronic infection
- Should only be ordered if chronic HBV is established
- Detection of HBeAg in serum indicates active viral replication, high level of infectivity, helpful marker for treatment
- Loss of HBeAg, appearance of anti-HBe indicates conversion to non-replication or mutation (Asian population)
Monitoring Acute HBV

• Conventionally, HBsAg and anti-HBs have been recommended monthly

• If, after 6 months, HBsAg is still positive, the patient has chronic infection
Algorithm for Follow-up of Chronic HBV Infection

A Management of Chronic HBV Infection*

HBsAg +

HBeAg

Positive

ALT < 1 X ULN

Q 3-6 mo ALT
Q 6-12 mo HBeAg

ALT 1-2 X ULN

Q 3 mo ALT
Q 6 mo HBeAg
Consider biopsy if persistent or age > 40, Rx as needed

ALT >2 X ULN

Q 1-3 mo ALT, HBeAg
Treat if persistent
Liver bx optional
Immediate Rx if jaundice or decompensated

* HCC surveillance if indicated
Algorithm for follow-up of Chronic HBV Infection

Management of Chronic HBV Infection*

HBsAg +

- HBeAg
  - Negative
    - ALT ≥ 2X ULN
      - HBV DNA ≥ 20,000 IU/mL
        - Treat if persistent,
          Liver biopsy optional
    - ALT 1-2X ULN
      - HBV DNA 2,000-20,000 IU/mL
        - Q 3 mo ALT & HBV DNA
          Consider biopsy if persistent
          Rx as needed
    - ALT < 1X ULN
      - HBV DNA < 2,000 IU/mL
        - Q 3 mo ALT X 3,
          Then Q 6-12 mo
          if ALT still <1x ULN

* HCC surveillance if indicated
# Hepatitis B Test Interpretation

## Serologic Marker

<table>
<thead>
<tr>
<th>HBsAg</th>
<th>Total anti-HBC</th>
<th>IgM anti-HBC</th>
<th>Anti-HBs</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>No evidence of exposure or immunization</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>chronic infection</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>acute infection</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>recovered &amp; immune</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>immune</td>
</tr>
</tbody>
</table>

## Atypical Result Patterns

- Passively acquired antibodies from blood transfusion
- Escape mutants
  - surface antigen
  - e antigen
- Recent immunization
Diagnosis of HCV Infection

• Usually not suspected until
  ➢ patient donates blood, has + anti-HCV
  ➢ patient has chemistry testing performed for the flu or other mild illness and is found to have a high ALT test – additional testing shows a positive anti-HCV

• Thus, almost all patients who need a diagnostic workup will have a positive anti-HCV test
Natural History of Hepatitis C

- **Acute Hepatitis C**
  - Symptoms are rare
  - Mostly undiagnosed

- **Chronic Hepatitis C**
  - 85% of cases

- **Cirrhosis**
  - 20% of cases

- **Liver cancer**
  - 10% of cases

- **In the US**
  - 4 million infected
  - 10,000 deaths/year

#1 cause for liver transplant in the US
Risk Factors - HCV Transmission

- Injection Drug Users: 60%
- Sexual: 15%
- Health Care Workers: 5%
- Non Identified Risks: 10%
- Transfusions (before screenings): 10%
- Organ transplants: 10%
- Perinatal: 5%

Source: Centers for Disease Control and Prevention
Tests for HCV

- Anti-HCV antibodies
  - screening test (EIA or CIA)
  - recombinant Immunoblot Assay (RIBA)

- HCV RNA
  - qualitative PCR
  - quantitative bDNA
  - quantitative real-time PCR
HCV Immunoassay (IA)

HCV IA detects antibodies to 3 or more viral proteins.
Hepatitis C Virus (HCV)

Genome and proteins

9900 base pairs

message-sense RNA

5'

NS1 NS2 NS3 NS4 NS5
C S NS1 NS2 NS3 NS4 NS5

c22-3 gp33 gp70 c33c c100-3 5-1-1

Structural proteins

Non-structural proteins

polymerase
HCV Diagnostic Algorithm

High Positive anti-HCV

↓

HCV RNA

→ Pos

Currently infected

↓

Neg

anti-HCV by RIBA

→ Neg

Never infected

↓

Pos

Infected, but recovered
HCV Diagnostic Algorithm

Low Positive anti-HCV

\[\text{anti-HCV by RIBA} \quad \begin{cases} \text{Neg} & \text{Never infected} \\ \text{Pos or indeterminate} & \text{Currently infected} \end{cases} \]

\[\text{HCV RNA} \quad \begin{cases} \text{Pos} & \text{Currently infected} \\ \text{Neg} & \text{Not currently infected, could have recovered} \end{cases} \]
HCV Points

• Acute HCV is rarely diagnosed (<20%)

• 85% of individuals that are infected with HCV become chronically infected (do not clear the virus)

• High positive anti-HCV screens are true positives (>95%) and have active infection (90%)

• Low positive anti-HCV screens need to be confirmed

• RIBA
  ➢ can be used to confirm low positive anti-HCV screens
  ➢ can distinguish between infection (past/present) and a false positive screen
  ➢ cannot discriminate between active and resolved infection
  ➢ should not be used to confirm high anti-HCV screens

• Only NAT testing can determine if a patient has active infection