Viral Testing – Hepatitis and HIV

Christopher Doern, PhD D(ABMM)
UT Southwestern Medical Center
Dallas, TX
Learning Objectives

• After this presentation you should be able to...

1. Discuss the clinical presentation of Hepatitis disease.
2. Understand the proper methods for diagnosing and monitoring viral hepatitis.
3. Discuss the clinical presentation of HIV.
4. Understand the proper methods for diagnosing HIV and monitoring infection.
Hepatitis A

- Hepatitis A – within the family *Picornaviridae*
  - Small, non-enveloped virus
  - Robust virus – survives low pH and has remarkable thermostability (especially in the presence of magnesium salts)
- Two major genotype groups found in humans
- Tropism for the liver
- Fecal transmission
Clinical Presentation – Hepatitis A

• Spread by having close contact or by eating food or drinking water containing HAV
• Causes a “flu-like” illness
• Jaundice (yellow skin or eyes, dark urine)
• Severe stomach pains and diarrhea (most in children)
  – 1 in 5 is hospitalized (3-6 deaths per 100,000)
  – Adults often too ill to work for up to a month
Prevention and Treatment – Hepatitis A

• Vaccine exists
  – Vaccination efficacy not impacted by strain differences

• Who should get it?
  – Kids between 12-23 months of age
  – >1 year and traveling to places of high prevalence
  – MSM’s
  – IV drug users

• Not eating poop.
  – Good general rule of thumb

• No treatment

Diagnosis of Hepatitis A

- **Diagnosis not complicated by strain differences.**
- **Serology**
  - Anti-HAV IgM documented in the acutely or recently ill patient
  - 4 fold rise in anti-HAV titer in paired sera
  - A single total anti-HAV does not have diagnostic value because once positive it is positive for life
    - It does indicate immunity though and can be used for prevaccination screening
  - Generally a good test but high false positive rate if ordered in wrong setting
- **PCR**
  - Stool or blood of acutely infected
  - Not commonly used

### Hepatic Function

- **Alkaline phosphatase**
- **Aspartate aminotransferase (AST)**
- **Alanine aminotransferase (ALT)**
- **Bilirubin, direct**
- **Protein, total**
- **Bilirubin, total**

University of Washington, Seattle Website - http://depts.washington.edu/labweb/Divisions/Viro/Hepatitis_sero.htm
What a clinical chemist should know about diagnosing HAV

• Technology for HAV IgM is very well developed
  – Not much difference between the methods

• Choices for which test to offer could be based on lab convenience
  – Single-strip enzyme linked immunosorbent assay (ELISA)
    - small volumes
  – Complete automation

• Usually done from Serum but saliva can be used in research settings

• Test Principles
  – All assays use inactivated whole HAV

Test Principles: IgM class capture and Indirect ELISA
Hepatitis E

- Hepatitis E – within the family *Hepeviridae*
  - Single stranded RNA
  - non-enveloped virus
  - Less stable than HAV
    - Sensitive to freeze-thaw and more thermolabile than HAV
    - Probably the reason for low secondary attack rate.

- Great genetic diversity – four genotypes

- Transmission
  - Fecal-oral transmission
  - Eating meat of deer, boars and pigs
  - Perinatal HEV transmission from infected mothers is common.
Clinical Presentation – Hepatitis E

• Fever
• Loss of appetite
• Jaundice (yellow skin or eyes, dark urine)
• Abdominal pain
• Acute infection usually self-limited
• Pregnant women may progress to liver failure and there is a risk of spontaneous abortion or premature delivery
Prevention and Treatment – Hepatitis E

• No Vaccines exists
• Again, don’t eat poop.
• Travelers to endemic areas should avoid drinking unboiled or unchlorinated water as well as ice.
• Thoroughly cook meets
• No treatment - supportive

Diagnosis of Hepatitis E

- Genetic diversity has an impact on sensitivity of serologic assays.
- Serology
  - Anti-HAV IgM documented in the acutely or recently ill patient
  - Genotypic strain differences result in great assay to assay variability in sensitivity
  - Assay performance also differs depending on the antigen used in the assay
  - IgG of little use because especially in places where HEV is endemic
    - More useful in low prevalence areas
- PCR
  - Exhibits significant viremia which can be detected with reverse transcriptase PCR at the time of disease
  - Not commonly used, but may be useful in confirming indeterminate serological results
  - Long term detection of HEV RNA in stool is suggestive of chronic infection

http://pathmicro.med.sc.edu/virol/HEPE-CD1.gif

What a clinical chemist should know about diagnosing HEV

- Technology for HAV IgM is improving
  - IgM preferred for diagnosis
- IgM ELISAs now used and based on highly conserved and immunodominant capsid protein
  - ORF2.1 antigen
- Some assays target r55K antigen
- IgA may be of use but no commercially available tests

- Test Principles
  - Use protein expressed from *E. coli*.
  - IgM class capture and indirect ELISAs are used
Hepatitis B

• Hepatitis B – within the family *Hepeviridae*
  – Circular partially double stranded DNA
  – Enveloped virus
  – >2 Billion people infected world wide
    • Most in Asia and Africa
    • ¼ of children will die as adults from liver cirrhosis or hepatocellular carcinoma (HCC)
    • Up to 1.4 million infected in the US

• Transmission
  – Vertical (mother to child perinatally)
  – Exposure to HBV containing blood or body fluids (~30% transmission rate)
  – Close contact with infected in early childhood
  – Sexual transmission
  – IV Drug use
  – NOT transmitted by
    • Casual contact
    • Fecal oral
Clinical Presentation – Hepatitis B

• >5 years old
  – 30-50% develop symptoms following exposure

• 60-150 day incubation period

• Usually asymptomatic in kids <5 and immunocompromised

• Acute -> Chronic
  – 30-90% of infected infants progress to chronic disease

• Acute
  – Early symptoms
    • Malaise
    • Fatigue
    • Anorexia
  – Late symptoms
    • Jaundice
    • Nausea
    • Vomiting
    • Abdominal pain

• Chronic
  – Liver cirrhosis
  – HCC
Prevention – Hepatitis B

• Vaccine exists and is effective
• First example of vaccine prevention of cancer
• Can be used a post-exposure prophylaxis in exposed non-immune individuals
  – Administer within 12 hours
• 2 Forms of vaccine
  – Plasma derived – Used in Asia
  – Recombinant
    • Anti HBSurfaceAg is sufficient for protection so most recombinant vaccines include on HBSurface Antigen

Principles and Practices of Infectious Disease: Mandel 6th Ed. – Chapter 142
Treatment of Hepatitis B

**Acute**
- Supportive
- Avoid medications metabolized by the liver if possible
- Liver transplant for those with liver failure
- Antivirals are not indicated in acute disease

**Chronic**
- Goal to suppress viral replication

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**TABLE 142-5** Selection of Patients for Treatment in Chronic Hepatitis B

<table>
<thead>
<tr>
<th>HBeAg</th>
<th>HBV DNA*</th>
<th>ALT*</th>
<th>Treatment Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>&lt;2 × ULN</td>
<td>Observe patient, consider treatment if ALT elevated</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>&gt;2 × ULN</td>
<td>Treatment with IFN or LAM</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>&gt;2 × ULN</td>
<td>Long-term treatment</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>&lt;2 × ULN</td>
<td>If receiving LAM: change to ADV</td>
</tr>
<tr>
<td>+/-</td>
<td>+</td>
<td>Cirrhosis</td>
<td>No treatment</td>
</tr>
<tr>
<td>+/-</td>
<td>+</td>
<td>Cirrhosis</td>
<td>Compensated: IFN or LAM</td>
</tr>
</tbody>
</table>

*Typically arbitrarily defined as >10^5 copies/mL but may be lower in hepatitis B early antigen positive and cirrhosis.

May also use moderate to severe necroinflammation on liver biopsy as guide.

ADV, adeovir dipivoxil; ALT, alanine aminotransferase; HBeAg, hepatitis B early antigen; HBV, hepatitis B virus; IFN, interferon-alfa; LAM, lamivudine; ULN, upper limit of normal.

Adapted from AASLD Practice Guidelines (Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology. 2001;34:1225-1241).

Diagnosing Hepatitis B Infection

• Primary diagnosis
  – Based on HBV antibody and antigen detection (see next slide for interp)

• Monitoring of chronic
  – Serology – seroconversion
  – Antigen detection – persistence of HBSurfaceAg > 6 months defines chronic
  – HBVeAg used to be commonly measured as marker of viral shedding
  – HBV DNA – replaced HBVeAg as a marker of disease progression
What is the utility of HBV PCR

How it should be used

• To confirm positive or ambiguous serological results
• To set a baseline and to establish when treatment should begin
• For monitoring progression of disease
• For monitoring response to therapy

Interpretation of Values

• High values correlate with an increased risk of fibrosis and/or a progression to hepatocellular carcinoma
• Low values do not exclude the possibility of progressing to disease
• There is no threshold for defining someone who will progress to cirrhosis

Liaw and Chu. Lancet. 2009. 373
Valsamakis. Clin Micro Reviews. 2007. 20
How to Interpret HBV DNA Values

• Initial evaluation of disease
  – Part of algorithm when to start therapy
  – A threshold of >20,000 IU/mL is associated with active viral replication

• During therapy
  – 3 month intervals if on lamivudine, 6 month intervals if on other reverse transcriptase inhibitors or interferon alpha
  – A log increase in viral load while on therapy indicative of resistance

• At end-point of therapy
  – 12 month intervals to access a sustained virologic response or the necessity to reinitiate therapy
Serologic - Diagnosis of Hepatitis B

TABLE 142-3 Interpretation of Serologic Tests in Hepatitis B

<table>
<thead>
<tr>
<th>Test</th>
<th>Acute Hepatitis B</th>
<th>Immunity through Infection*</th>
<th>Immunity through Vaccination</th>
<th>Chronic Hepatitis B</th>
<th>Chronic Infection with Precore Mutant</th>
<th>Healthy Carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HBsAg</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anti-HBc</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Anti-HBe</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IgM anti-HBc</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HBV DNA†</td>
<td>Elevated</td>
<td>Normal</td>
<td>Normal</td>
<td>Elevated</td>
<td>Elevated</td>
<td>Normal</td>
</tr>
<tr>
<td>ALT</td>
<td>Elevated</td>
<td>Normal</td>
<td>Normal</td>
<td>Elevated</td>
<td>+</td>
<td>Normal</td>
</tr>
</tbody>
</table>

*Occasionally individuals with past infection have isolated anti-HBc only. The presence of an isolated IgG anti-HBc may indicate a window period during acute infection or remote prior infection with loss of HBsAg or anti-HBs. In such cases, an HBV DNA test may prove useful.

†Presence of HBV DNA depends upon the sensitivity of the test used.

ALT, alanine aminotransferase; HBc, hepatitis B core; HBc, hepatitis B early; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IgM, immunoglobulin M.
Serologic - Diagnosis of Hepatitis B

- **HBSurfaceAb**
  - Marker of immunity due to vaccination or seroconversion and clearance of past infection
- **HBcoreAb IgM**
  - Marker of acute infection
- **HBcoreAb Total**
  - Indicates past or current infection
- **HBsAg**
  - Detection in serum establishes the diagnosis of infection and indicates high infectivity

- **HBeAg**
  - Detection in serum indicates rapid viral replication and usually correlates with high viral loads
  - Patients with high viral loads and no HBeAg may indicate pre-core mutation
Other things a clinical chemist should know about diagnosing HBV

• Numerous commercial tests available
  – Too many to discuss here
• You will encounter this testing primarily as an acute hepatitis panel
  – Mayo and ARUP panels include
    • HAV IgM, HBV core Ab
    • IgM, HBV Surf Ag, HCV Ab
• Susceptibility testing by Sequencing available
  – Some recommend testing after a 1-Log10 increase from lowest HBV DNA load in the face of anti-HBV therapy
Hepatitis C

- Hepatitis C within the *Faviviridae* family
  - Enveloped, single stranded, positive sense RNA virus
  - So far has not been grown in cell culture
  - Classified into 6 major genotypes which may differ in sequence homology by up to 30%.
    - These have epidemiological significance
      - For Example – 71.5% of US infections are genotype 1

- Transmission
  - Growing concern in US – 3.8 million infected
    - 170 million world wide
  - Infects primarily humans.
  - Primarily transmitted from human to human through blood exposure
    - IV drug use most common (>50%)
  - NOT efficiently transmitted through sexual intercourse
  - Can be detected in saliva but casual contact with saliva is thought to be very low risk
  - Pregnancy NOT contraindicated
    - Not transmitted through breast milk
Clinical Presentation – Hepatitis C

• Acute infection
  – Typically asymptomatic
• Chronic infection
  – Develop systemic symptoms
    • Cryoglobulinemia involving the skin, kidneys and nervous system
    • Sjogren syndrome – disorder of immune system characterized by dry eyes and mouth
    • Non-Hodgkin lymphoma
    • Cirrhosis (20%)
    • Hepatocellular carcinoma (1-5%)
Prevention – Hepatitis C

- No vaccine is available
- Avoid IV drug use
- Be careful when getting tattoos
- Although sexual transmission is rare it is best to avoid unprotected sex with infected individuals

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Treatment of Hepatitis C

- Understanding of epidemiology important for individualizing therapy
  - HCV – 1b most common worldwide

- Primary goal of treatment is sustained virologic response
  - < 50 IU/ml at the end of a 24 week follow-up after cessation of treatment

*Figure 2* Patterns of on-treatment and off-treatment virological responses. PegIFN, peginterferon; RBV, ribavirin; RVR, rapid virological response; cEVR, complete early virological response; pEVR, partial early virological response; SVR, sustained virological response; EOT, end of treatment; detection limit of HCV RNA, 50 IU/mL.
## Treatment of Hepatitis C

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>Either Pegylated Interferon alfa 2a (PEG-IFN) alone</td>
</tr>
<tr>
<td>2,3 and 4</td>
<td>PEG-IFN PLUS Ribavirin</td>
</tr>
<tr>
<td>1</td>
<td>PEG-IFN PLUS Ribavirin PLUS Teleprevir/Bocepr eivr</td>
</tr>
</tbody>
</table>

![Graph showing sustained virologic response by genotype, fibrosis, HCV RNA, age, gender, and weight.](image-url)
Diagnosing Hepatitis C Infection

• Patients that should be considered for testing
  – New onset jaundice
  – Abnormal liver enzyme levels
  – Recipient of blood products prior to 1991
  – Hemophilliac
  – IV Drug user

• Diagnostic methods
  – Antibody screens
  – HCV RNA detection by PCR from serum

• Approach differs for acute versus chronic
Diagnosing Acute Hepatitis C Infection

• Diagnosis of disease primarily made with serology and confirmed with PCR
  – Screen usually part of an acute hepatitis panel (discussed previously)
• Seroconversion happens within 8 weeks
• Antibody screening recommended 4-6 months following exposure

TEST METHOD
• Screen for antibody against HCV NS3, NS5 and core antigens with 3rd generation EIA’s
  – These antigens are targeted by the immune system in early response
• Positives usually confirmed with either...
  – Nucleic acid test
  – Repeat testing
  – RIBA (recombinant immunoblot)
    • Not commonly used anymore
The RIBA

• Was used to confirm positive antibody screens when early generation serologic tests were non-specific.

• Negative RIBA suggest false positive screening result
  – May consider follow-up testing (PCR) because this could also be in the acute phase of infection

• Positive results confirm infection

• Indeterminate are problematic because they cannot exclude infection
  – Proceed as with negatives to confirm

• Based on Western blot
  – HCV recombinant antigens and peptides attached to membranes
  – Membrane strips incubated with patient serum or plasma
  – Peroxidase-labeled anti-human IgG used for detection
  – Band intensity scored as high (level II) and low (level I)

Principle of EIA Screening WB/RIBA confirmation
• EIA - includes multiple antigens in one read-out but positive results do not distinguish between single antigen and multiple antigen detection.
  - Efficient screening assay because majority are negative.
• WB/RIBA – evaluates number of antigens detected.
Managing Chronic Hepatitis C Infection

- Quantitative PCR assays vary from laboratory to laboratory
- Only linear in log10 format
  - Difficult to grasp and differs from most other quantitative assays
- Interpretation
  - Values can vary up to 10-fold in stable individuals with untreated chronic hepatitis
  - Therefore changes of less than 10-fold may not be clinically significant

TEST METHOD
Quantitative PCR
- Many different methods
- RNA virus requires reverse transcription
  - Most target 5’ UTR

Genotyping
- Useful for counseling patients on likelihood of successful treatment
- Based on 5’ UTR sequences
- Subtype analysis most accurately generated from NS5b, core and core-E1 genes
- Direct sequencing considered the gold standard
Human Immunodeficiency Virus (HIV)

- Etiologic agent of AIDS
- Enveloped plus-stranded RNA virus
- Member of the family Retroviridae
- Two major viral species
  - HIV-1 – more virulent and responsible for the world wide AIDS pandemic
  - HIV-2 – less pathogenic and a more limited geographic distribution
Clinical Presentation – HIV

• Initial infection presents with flu-like illness that may last for a few weeks
  – Particularly infectious during this period
• HIV progresses to AIDS (~10 years if untreated)
  – Susceptible to opportunistic infection
• Transmission
  – Sexual contact
  – Exposure to contaminated body fluids such as blood, semen and vaginal secretions
    • Risk of transmission following occupational exposure ~0.3%
  – Mother to child transmission
    • In utero, during labor and delivery, during breast feeding
Prevention and Treatment – HIV

• Vaccine does not exist
• Avoid exposure to contaminated products
• Protected sex
• Antiviral treatment can lower viral load in pregnant women and reduce risk of fetal transmission.
• Antiviral Targets
  – Integrase inhibitors
  – Entry and Fusion Inhibitors
  – Protease inhibitors
  – Non-nucleoside reverse transcriptase inhibitors
  – Nucleoside reverse transcriptase inhibitors


http://aidsinfo.nih.gov/drugs/#
Diagnosis of HIV – Waived Test

- In home test – waived
- Lateral flow assay that detects antibody against HIV 1 and 2
- Conducted from swab of gums
  - Sensitivity = 99.3%
  - Specificity = 99.8%
- Now FDA approved for over the counter use.
Diagnosis of HIV – Screening Assays

- Time between infection and detection = Window period (see next slide)
- Generation 1-3 EIA
  - Relied on antibody only
- Generation 4 EIA
  - Combine antibody with p24 antigen detection

- Point of Care Assays
  - Immunochromatographic
    - Lateral flow assays that are extremely easy to perform
  - Performance is very good
    - Equal to that of older generation EIA’s
    - Trial data is very good. No studies yet on how it will perform in the hands of the non-laboratorian
Using HIV Ag/Ab Combo assays closes the window to detect HIV infection up to 5 days.
Confirmatory Tests

**Western Blot**
- Considered the gold standard
  - Supplied in kit form with HIV antigens pre-spotted to the membrane
  - Testing laboratory incubates the membranes with patient serum.
  - Develop reaction with enzyme labeled anti-human antibody

**DNA PCR**
- Use PCR to detect proviral DNA

HIV-1 Viral Load Assays

- Measure HIV-1 RNA in plasma
- Goal of therapy is to reduce viral loads to undetectable
- 3 FDA cleared assays
  - Not reliable for HIV-2
- Target conserved gag gene.
- Used to monitor the response to therapy and/or progression of disease
- Genotyping assays look for the presence of Resistance mutations.

Calculation

- Compared to RNA standards that range from 400-750,000 copies/ml
- Or 50-100,000 copies/ml
  - This includes higher input volume and ultracentrifugation

Guo. 2010. Lab Medicine
In the course of a health care worker needle stick, which blood borne pathogen is most likely to be transmitted?

a. HBV  
b. HCV  
c. HIV  
d. HAV

Which of the following viruses has a vaccine that can prevent it?

a. HBV  
b. HCV  
c. HIV  
d. HEV

4th generation EIA’s for the diagnosis of HIV rely on which combination for diagnosis?

a. Antibody and DNA  
b. Antibody and RNA  
c. P24 Surface and DNA  
d. Antibody and p24 antigen