Overview

Part A: GENERAL SEROLOGY
  - IgG vs IgM
  - Definitions
  - The biochemists view of Immunoassays
  - The serologists view of Immunoassays
  - Common assays

Part B: HEPATITIS B
  - What is hepatitis
  - History of Hep B
  - Clinical Presentation
  - Measurement

Part C: CASE STUDY
PART A: GENERAL SEROLOGY
**HUMOURAL IMMUNE RESPONSE**

**Primary**
- Appearance of IgM
- Within 3 to 5 days following exposure
- IgG production follows within the first week

**Secondary / Amnestic**
- Mediated by IgG
- Response earlier c.f. primary
- Typical response within 3 days
- Greater abundance than in a primary response

Diagram from: [http://coursewareobjects.elsevier.com/objects/ek/Wyllie/pediatricGI3eFC/images/063002.jpg](http://coursewareobjects.elsevier.com/objects/ek/Wyllie/pediatricGI3eFC/images/063002.jpg)
Stedman’s Serology Definitions

- **Serology**: The branch of science concerned with serum, especially with specific immune or lytic serums; to measure either antigens or antibodies in sera

- **Serologic test**: Any diagnostic test in which serum (blood) is used

- **Sero-negative**: Lacking an antibody of a specific type in serum; denoting absence of prior infection with a specific agent, disappearance of antibodies after treatment of a disease, or absence of antibody usually found in a given syndrome

- **Sero-conversion**: The process by which, after exposure to the etiologic agent of a disease, the blood changes from a negative to a positive serum marker for that specific disease

- **Sero-positive**: Containing antibody of a specific type in serum; denoting the presence of immunologic evidence of a specific infection or presence of a diagnostically useful antibody

- **Sero-epidemiology**: An epidemiologic study based on the detection of infection by serologic testing
IMMUNOASSAYS

Stedman’s definition:
“Detection and assay of substances by serologic (immunologic) methods”

These techniques use the interaction of Antigens (Ag) and Antibodies (Ab) to detect and/or measure a particular analyte.

Applications include:
- Hormones
- Metabolic Markers
- Cancer Markers
- Cardiac Markers
- Drugs (Therapeutic and Abused)
- Immunology
- Infective Agents

IMMUNOASSAY: e.g. Oestradiol
IMMUNOASSAY: e.g. Architect Oestradiol

Sample Containing Estradiol Bound to Sex Hormone Binding Globulin

Solid Phase Suspension Containing:
- a) Blocking Agent
- b) Microparticles Coated with Rabbit Monoclonal Anti-Estradiol Ab
- c) Specimen Diluent

Incorporate 5 min.

Estradiol Acridinium Tracer

Incorporate 21 min.

Blocking Agent

n Estradiol Bound to Solid Phase
n Blocking Agent Binds to SHBG to Prevent Estradiol Re-binding

Tracer fills unoccupied Ab binding sites

Add Pre-Trigger, Trigger Solutions

Wash (x4)

Read

Modified from slide provided courtesy of Abbott Diagnostics
Calibration of an Immunoassay

This is an example of a quantitative assay
What makes Serology assays different from Biochemical Immunoassays?

1. The target for a serology assay is very complex

2. These targets may possess the ability to “change”

3. Generally interested in presence or absence of the target, as opposed to a numerical measurement
CALIBRATING A QUALITITATIVE IMMUNOASSAY

- S/CO: Ratio of the value of the sample to the designated value of the cut-off
  - <1.0  “non-reactive”
  - =>1.0  “reactive”
WHAT DO S/CO NUMBERS MEAN?

- Signal to Cut-Off
- Affinity, Binding strength, Reactivity of Ab/Ag
- Actual quantity of Ab or Ag is usually not important for diagnosis
- Different antibody/antigen combinations can demonstrate different levels of binding
- Assay response to concentration of Ab or Ag is NON-LINEAR (no Immunoassay is linear)
- No Standard curve for Qualitative assays
QUALITATIVE v.s. QUANTITATIVE ASSAYS

• QUANTITATIVE assays
  – calibration curve relates signal to concentration

• QUALITATIVE assays
  – measures Rate Units or Relative Light Units etc. (i.e. reaction detectable above “noise”)

• S/CO values do NOT relate to Concentration of the Ab or Ag being assayed
• The area around the cutoff (usually at a S/CO of 0.8-1.2) where results deemed inconclusive
• Further testing required to determine reactive or non-reactive status
REPORTING TERMINOLOGY

• Reactive and Non-Reactive
  – Preferred terminology
  – Reporting results as Positive or Negative may have medico-legal implications

• Initial and repeat reactives
  – Initial Reactives = first pass test gives a reactive result
  – Repeat Reactives = subsequent tests using the same assay are also reactive
CONFIRMATION TESTING

• Reactive and Greyzone results may need to be confirmed
  • Same method, perhaps with sample manipulation
  • Alternate method
  • Reference Lab
SENSITIVITY & SPECIFICITY: Biochemist’s definitions

• Functional Sensitivity – lowest concentration measurable at a defined CV (20% for TSH)

• Analytical Sensitivity – lowest concentration distinguishable from zero

• Specificity – measure of an assay’s cross-reactivity with similar molecules
SENSITIVITY & SPECIFICITY: Serologist’s definitions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>Proportion of TRUE POSITIVES Identified by the Test</td>
<td>POS Results / True Positives</td>
</tr>
<tr>
<td>Specificity</td>
<td>Proportion of TRUE NEGATIVES Identified by the Test</td>
<td>NEG Results / True Negatives</td>
</tr>
</tbody>
</table>

- NOTE: These parameters are compared to an existing “gold standard” assay and therefore relate purely to a comparison between assays

QC for Qualitative Serology Assays

**Lot to Lot Variability:**
- Assay-specific range for S/CO is established over many master lots of REAGENTS and CONTROLS
- Variability can be due to variations in antibody source, raw material variations e.g. micro-particles etc

**Interpreting QC:**
- Sensitivity of each lot is NOT related to S/CO value – higher S/CO on one batch does NOT mean that batch detects “MORE ANTIBODY” (Assays are non-linear)
- Sensitivity is validated by use of low positive samples at manufacture
- S/CO values for controls may vary lot to lot

**Control Charts:**
- Mean values for controls may shift between lots
- This will effect running mean, SD, and CV calculations
- Ideally archive control data prior to starting a new lot to avoid problems
A GOOD SEROLOGY ASSAY

• Sensitivity and Specificity
• Sero-conversion Panels Discrimination
• Detection of all variants
• Meets regulatory guidelines
• Initial Reactives = Repeat Reactives
• No Prozone
• Consistent manufacture
• Check for FDA approval etc
Clinical Applications

- Syphilis
- Peptic ulcer disease
- HIV (human immunodeficiency virus)
- Congenital Infections i.e. TORCH
  - Toxoplasma, Rubella, Cytomegalovirus, Herpes
- Capsular polysaccharide antigen response
- Viral Hepatitis
PART B: HEPATITIS B
## Acute Hepatitis: Differential Diagnosis

= Inflammation of the Liver

<table>
<thead>
<tr>
<th>TYPE</th>
<th>CAUSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral Hepatitis</td>
<td>Hepatitis A, B, C, D, E</td>
</tr>
<tr>
<td></td>
<td>CMV, EBV, HSV, VZV, yellow fever</td>
</tr>
<tr>
<td>Bacterial Hepatitis</td>
<td>Typhoid fever, Q fever, RMSF, leptospirosis, secondary syphilis, sepsis</td>
</tr>
<tr>
<td>Parasitic Infections</td>
<td>Toxocanasis, liver flukes</td>
</tr>
<tr>
<td>Drugs</td>
<td>ASA, acetaminophen, INH, rifampin, oral contraceptives, anti-seizure medications, carbenicillin, sulfonamides</td>
</tr>
<tr>
<td>Toxins</td>
<td>Alcohol, carbon tetrachloride</td>
</tr>
<tr>
<td>Autoimmune Diseases</td>
<td>Autoimmune hepatitis, SLE</td>
</tr>
</tbody>
</table>
# Viral Hepatitis: Clinical Manifestations

## Symptoms
- Malaise: 76-94%
- Anorexia: 71-96%
- Dark Urine: 65-94%
- Nausea: 61-81%
- Abdominal pain: 26-68%
- Scleral icterus: 48%
- Vomiting: 20-37%

## Signs
- Jaundice: 70-90%
- Hepatomegaly: 14-69%
- Tender liver: 20-86%
- Rash: 40%
- Splenomegaly: 3-21%
- Fever: 1-8%
- High LFT’s: 100%

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Asymptomatic ➔ Symptomatic ➔ Fulminant liver failure ➔ Death
<table>
<thead>
<tr>
<th>Virus</th>
<th>Hepatitis A</th>
<th>Hepatitis B</th>
<th>Hepatitis C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmission route</td>
<td>Faecal - oral</td>
<td>Infected needle or blood, sexual contact</td>
<td>Infected needle or blood, sexual contact</td>
</tr>
<tr>
<td>Incubation time (acute infection)</td>
<td>15 – 50 days</td>
<td>45 – 160 days</td>
<td>14 – 180 days</td>
</tr>
<tr>
<td>Onset</td>
<td>Sudden</td>
<td>Either sudden or slow, unnoticed</td>
<td>Usually slow unnoticed</td>
</tr>
<tr>
<td>Severity</td>
<td>Mild</td>
<td>Occasionally severe</td>
<td>Usually slow developing and symptoms not specific or strong</td>
</tr>
<tr>
<td>Chronic Form</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Associated with other diseases</td>
<td>None</td>
<td>Liver cancer, cirrhosis</td>
<td>Liver cancer, cirrhosis</td>
</tr>
<tr>
<td>Virus</td>
<td>Hepatitis A</td>
<td>Hepatitis B</td>
<td>Hepatitis C</td>
</tr>
<tr>
<td>-------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Testing to diagnose acute infection</td>
<td>HAV Ab IgM</td>
<td>HBsAg, Anti-HBc IgM</td>
<td>Anti-HCV, HCV RNA (note may have same results as in chronic hepatitis)</td>
</tr>
<tr>
<td>Testing to diagnose chronic infection or to monitor treatment</td>
<td>N/A</td>
<td>HBsAg, HBV DNA, HBeAg, anti HBe</td>
<td>Anti-HCV (once), HCV RNA or viral load, HCV genotype (once)</td>
</tr>
<tr>
<td>Tests that detect previous infection</td>
<td>HAV Ab IgG</td>
<td>Anti HBs, Anti HBc total</td>
<td>Anti HCV</td>
</tr>
<tr>
<td>Vaccine Available?</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Common Treatment</td>
<td>None</td>
<td>Chronic form - Interferon, entecavir, tenofovir, lamivudine, adefovir</td>
<td>Chronic form interferon, usually with ribavirin</td>
</tr>
</tbody>
</table>
HISTORY:
Hepatitis B – the “Australia Antigen”

- Hepatitis B virus = HBV
- Discovered in the early 1960s, using a blood sample from an Australian aborigine
- Led to the development of diagnostic assays for hepatitis B surface antigen (HBsAg)
- This continues to be a key marker for HBV infection today
HEPATITIS B (HBV): WORLD-WIDE

• Estimated 350 million people currently infected
• Worldwide prevalence is about 10-fold higher than the global AIDS cases
• About 80 million chronic carriers have progressed to hepatocellular carcinoma
• > 500,000 deaths occur each year
• Infection can be controlled by universal vaccination, passive immunization & recently antiviral therapy
• Early diagnostic detection plus infection-control measures have resulted in a significant reduction in HBV infection rates in developed countries
HEPATITIS B: THE VIRUS

- 3.2 kilobases
- Partially ss circular DNA genome
- HBV genome contains 4 genes with partially overlapping, open reading frames encoding 7 proteins:
  - Pol gene - Polymerase protein
  - C gene – Core (c) Ag and e Ag
  - S gene - large, medium, and small surface (s) Ag proteins
  - X gene - X protein
- HBV classified into 8 genotypes (A to H) based on a nucleotide divergence of approximately 25%
Table 1: Description of HBV Seromarkers and Their Diagnostic Outcome

<table>
<thead>
<tr>
<th>HBV Seromarker</th>
<th>HBV infection outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>Persistence for longer than 6 months can indicate chronic infection</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>Seroconversion to anti-HBs can indicate immunity</td>
</tr>
<tr>
<td>HBeAg</td>
<td>Determines relative infectivity</td>
</tr>
<tr>
<td>Anti-HBe</td>
<td>Seroconversion to anti-HBe indicates progression towards infection resolution</td>
</tr>
<tr>
<td>Anti-HBc IgM</td>
<td>Differentiates acute/recent infection from chronic carrier state or resolved HBV infection</td>
</tr>
<tr>
<td>Anti-HBc</td>
<td>Helps to establish the stage of chronic infection</td>
</tr>
<tr>
<td>HBV DNA</td>
<td>Persistence without seromarkers can indicate occult infection</td>
</tr>
</tbody>
</table>
TIME COURSE HEPATITIS B

ACUTE v.s. CHRONIC

Fig. 32-2. Serological events associated with hepatitis B virus (HBV) infection. A, Serological profile of acute hepatitis B infection with complete recovery; time in weeks. B, Serological profile of chronic hepatitis B infection; time in weeks, up to 52.

**HEPATITIS B: INTERPRETATION**

(https://www.cdc.gov/NCIDOD/DISEASES/HEPATITIS/b’faqb.htm)

Diagram from: Hepatitis B Virus - Methods in Clinical Analysis Kaplan et al 2009

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>negative</td>
<td>Susceptible</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>anti-HBs</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td>negative</td>
<td>Immune due to natural infection</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>anti-HBs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td>negative</td>
<td>Immune due to hepatitis B vaccination</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>anti-HBs</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td>positive</td>
<td>Acutely infected</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>IgM anti-HBc</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>anti-HBs</td>
<td>negative</td>
<td></td>
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<tr>
<td>HBsAg</td>
<td>positive</td>
<td>Chronically infected</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>IgM anti-HBc</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>anti-HBs</td>
<td>negative</td>
<td></td>
</tr>
</tbody>
</table>
Hepatitis B: Potential Outcomes

Initial infection

90%  <1%  *

10%  **

Resolution  Carrier  Chronic infection  Fulminant disease

Cirrhosis  Hepatocellular carcinoma

1%  HDV co- or superinfection may potentially accelerate or induce these outcomes

Role of mutant HBV strains postulated to potentiate these developments

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PART C: CASE STUDY

case from
http://www.cdc.gov/hepatitis/Resources/Professionals/Training/Serology/
CASE STUDY

Patient Information:

• A 43-year-old registered nurse was hired to work in the emergency room at a large tertiary care center
• She was given the 3-dose hepatitis B vaccine series followed by postvaccination testing two months after the last dose for antibody to hepatitis B surface antigen (anti-HBs)
• Her anti-HBs concentration was 5 mIU/mL

What does the anti-HBs result (5mIU/mL) indicate?
Case: What does the anti-HBs result (5mIU/mL) indicate?

• Option A: She is protected from HBV infection
  - Anti-HBs is the marker that indicates immunity to HBV infection
  - An anti-HBs result less than 10 mIU/mL within 1-2 months after completion of the hepatitis B vaccine series indicates that she is not protected against HBV infection

• Option B: She is infected with the hepatitis B virus
  - Anti-HBs is the marker that indicates immunity to HBV infection
  - An anti-HBs result less than 10 mIU/mL within 1-2 months after completion of the hepatitis B vaccine series indicates that she is not protected against HBV infection

• Option C: She is not protected from HBV infection
  - She is not protected from HBV infection
Case: What should be done next?

- Option A:
  - She should be revaccinated with a 3-dose hepatitis B vaccine series  

- Option B:
  - Nothing more should be done  

- Option C:
  - She should have the post-vaccination testing repeated  

ANSWER

- She should be revaccinated with a 3-dose hepatitis B vaccine series followed by post-vaccination testing for anti-HBs (1-2 months after the last dose)  
- 50-75% of people develop sero-protection after an additional series.
CASE

- She was revaccinated
- Her post-vaccination anti-HBs test result was 150 mIU/mL
- She is now protected from HBV infection
- The result was placed in her occupational health record

- Six years later, she had a needle-stick injury
- The source patient was HBsAg positive and anti-HCV positive

What needs to be done to protect her from HBV infection?
CASE: What needs to be done to protect her from HBV infection?

- **Option A:**
  - She should have a booster dose of vaccine

- **Option B:**
  - No postexposure prophylaxis is recommended

- **Option C:**
  - She should be tested for hepatitis B surface antigen

**ANSWER**

- No postexposure prophylaxis is recommended for persons who have ever had a documented anti-HBs result of at least 10 mIU/mL after hepatitis B vaccination, even if this result was many years in the past

- Immuno-competent persons who respond to hepatitis B vaccination remain protected even if the anti-HBs concentration falls below measurable levels
CASE: What needs to be done for the exposure to blood from an anti-HCV positive source patient?

• Option A: Nothing should be done

• Option B: Test only for ALT

• Option C: Baseline testing for anti-HCV and ALT

ANSWER

– Baseline testing for anti-HCV and ALT activity is recommended

– If an earlier diagnosis of HCV infection is needed, testing for HCV RNA by PCR may be performed at 4-6 weeks

– All positive anti-HCV results by enzyme immunoassay should be verified by supplemental testing with a recombinant immunoblot assay or PCR for HCV RNA
CASE: Should immune globulin be given?

Option A: Yes
Option B: No

ANSWER

Immune globulin is not effective for postexposure prophylaxis to prevent HCV infection.

In addition, antiviral agents (e.g., interferon) are not recommended for postexposure prophylaxis.

Follow-up: Baseline testing for anti-HCV was negative.
CASE: What additional follow-up should be done regarding her exposure to HCV-positive blood?

- Option A:
  - Provide follow-up testing for anti-HCV at 4-6 months after the needlestick

- Option B:
  - Provide counselling to refrain from blood donation until follow-up testing is done

- Option C:
  - Provide counselling to follow-up recommended infection control practices at work

- Option D:
  - All of the above

ANSWER

- Follow-up testing for anti-HCV and ALT testing at 4-6 months after the needle-stick should be done. Persons who are anti-HCV negative at 4-6 months can be assured that they did not become infected from the exposure.

- Persons who are exposed to HCV-infected blood should refrain from donating blood, plasma, organs, tissue, or semen during the follow-up period.

- No modifications to an exposed person's patient care responsibilities are necessary to prevent transmission. All health care professionals should follow recommended infection control practices to prevent blood exposures, including standard precautions and appropriate use of hand washing, protective barriers, and care in the use and disposal of needles and other sharp instruments.
CASE

• She was very relieved to find that her follow-up anti-HCV test was negative and her ALT level was within normal limits

• After this experience, she developed an in-service program for her hospital on safe use and disposal of needles and other sharp instruments
In summary

- Many serology assays are now run on automated platforms in biochemistry or core laboratories
- These assays are considered qualitative
- Calibration curves are different
- QC approach has some variations
- As Biochemists running Serology assays, we need to remember that:
  - Not all numbers have the same meaning
  - New assays are constantly being developed
  - CDC is a useful reference site