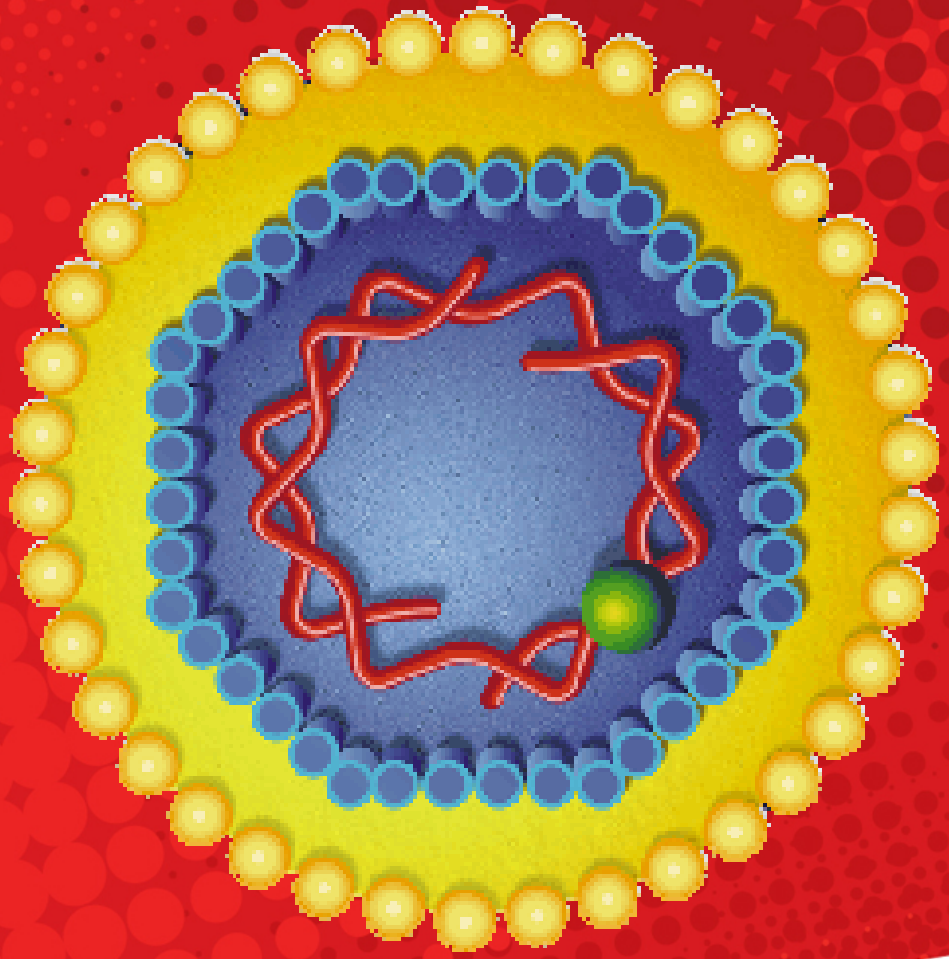
Serology for Biochemists



Dr Ronda Greaves

Diagram from: http://www.cdc.gov/hepatitis/Resources/Professionals/Training/Serology/training.htm

RMIT UNIVERSITY

Overview

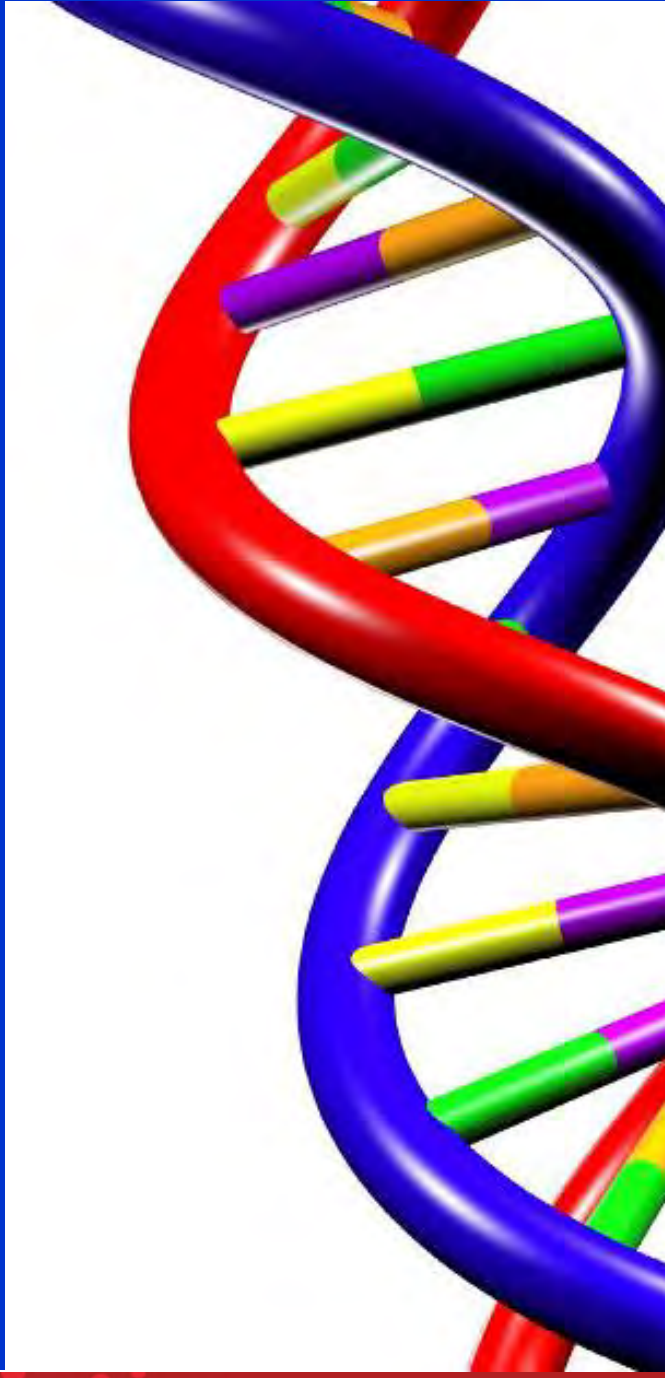
Part A: GENERAL SEROLOGY

- 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

Diagram from : http://coursewareobjects.elsevier.com/objects/elr/Wyllie/pediatricGI3e/IC/images/063002.jpg

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

Typical Ab response to exposure to Ag

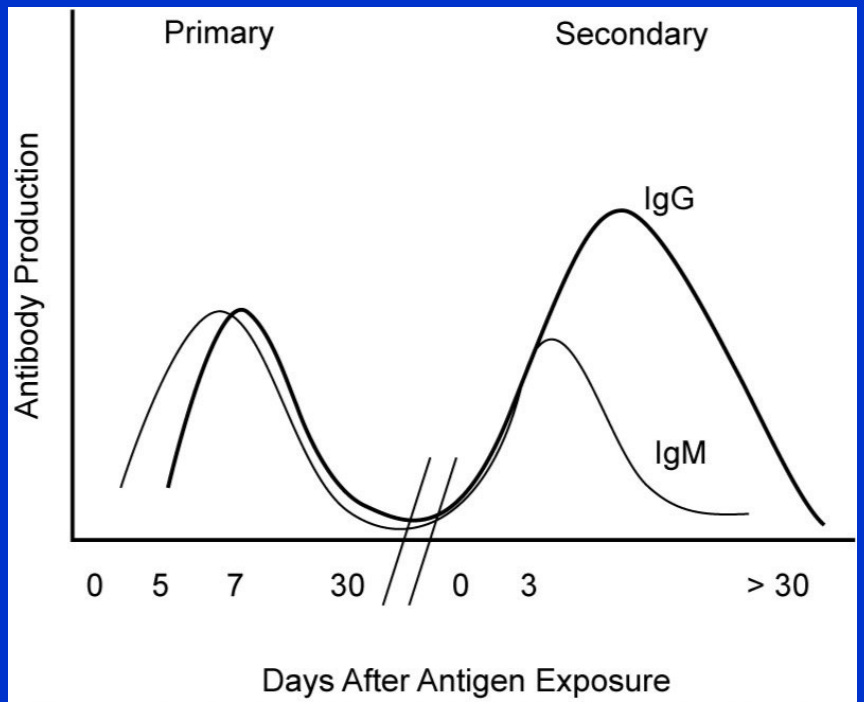


Fig. 10-1. Primary and secondary humoral immune responses. This figure illustrates the typical antibody response to exposure to antigen. Primary humoral immune responses are typified by the appearance of immunoglobulin (Ig)M within the first 3 to 5 days following exposure, followed by IgG production within the first week. Amnestic responses typically are mediated by IgG, which appears earlier (typically within 3 days) and in greater abundance than in a primary response.

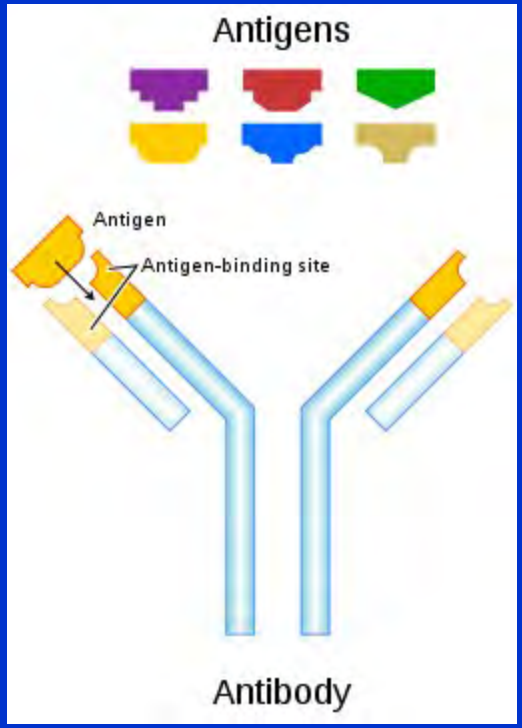
Stedman's Serology Definitions

- **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

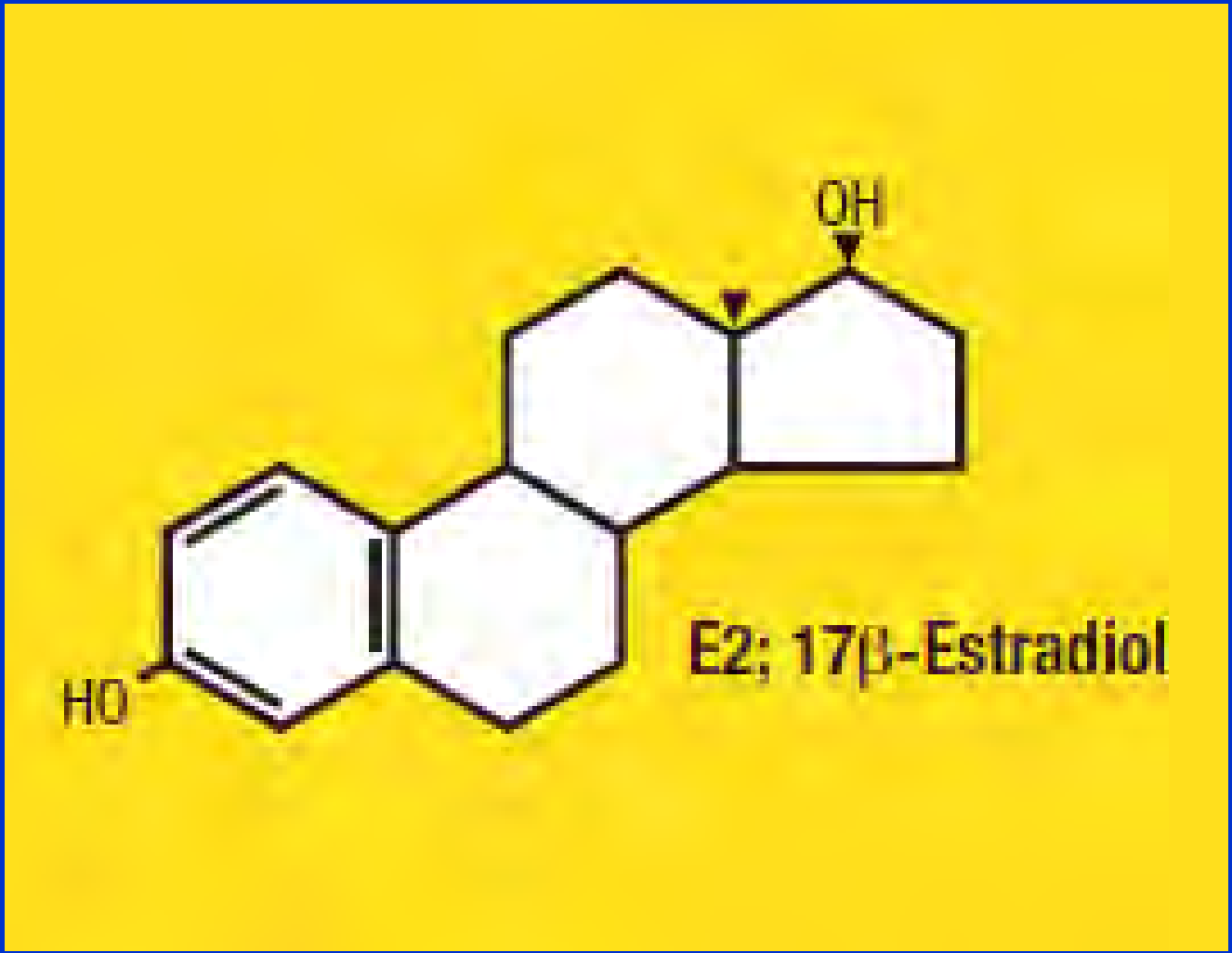
Stedman's definition:
"Detection and assay of substances by serologic (immunologic) methods"

- 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**

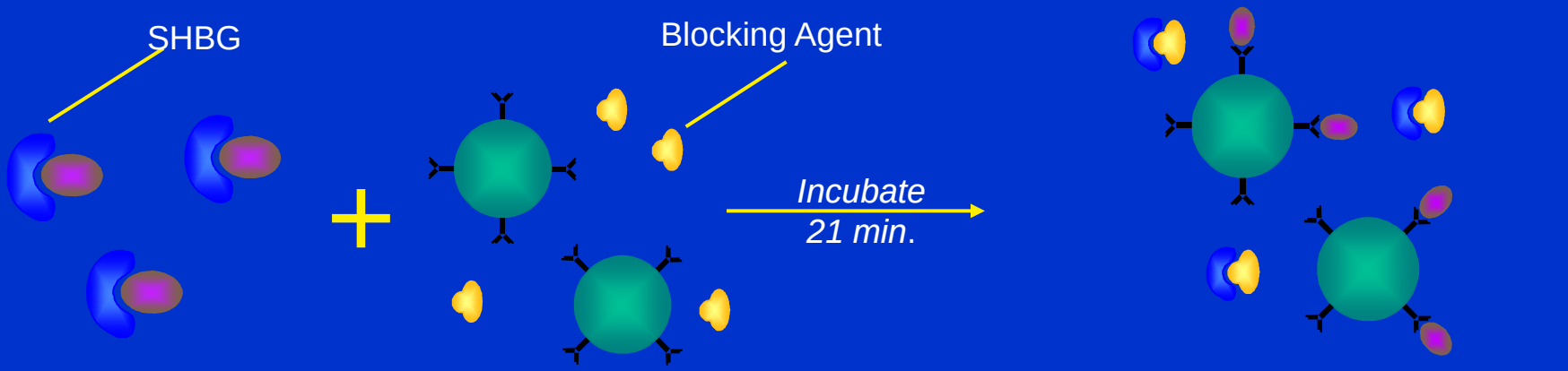


http://www.pharmaceutical-technology.com/features/feature63997/feature63997-3.html

IMMUNOASSAY: e.g. Oestradiol



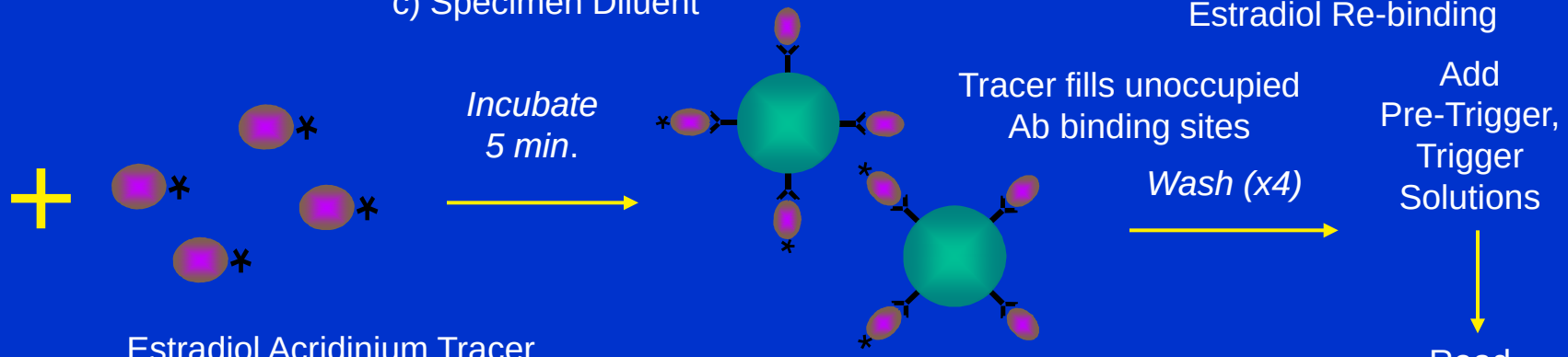
IMMUNOASSAY: e.g. Architect Oestradiol



Sample Containing Estradiol Bound to Sex Hormone Binding Globulin

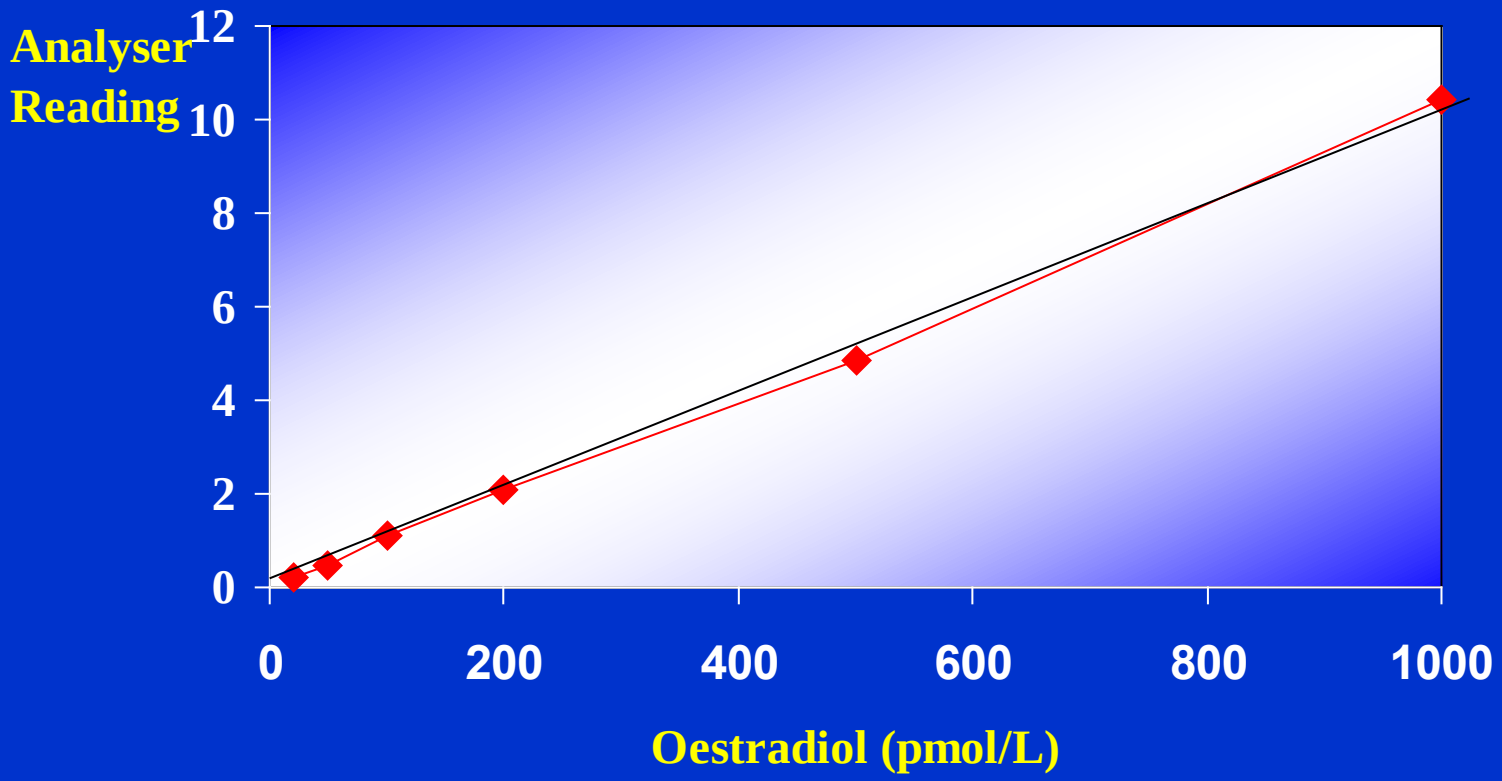
Solid Phase Suspension Containing:
a) Blocking Agent
b) Microparticles Coated with Rabbit Monoclonal Anti-Estradiol Ab
c) Specimen Diluent

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



Estradiol Acridinium Tracer

Calibration of an Immunoassay



This is an example of a quantitative assay

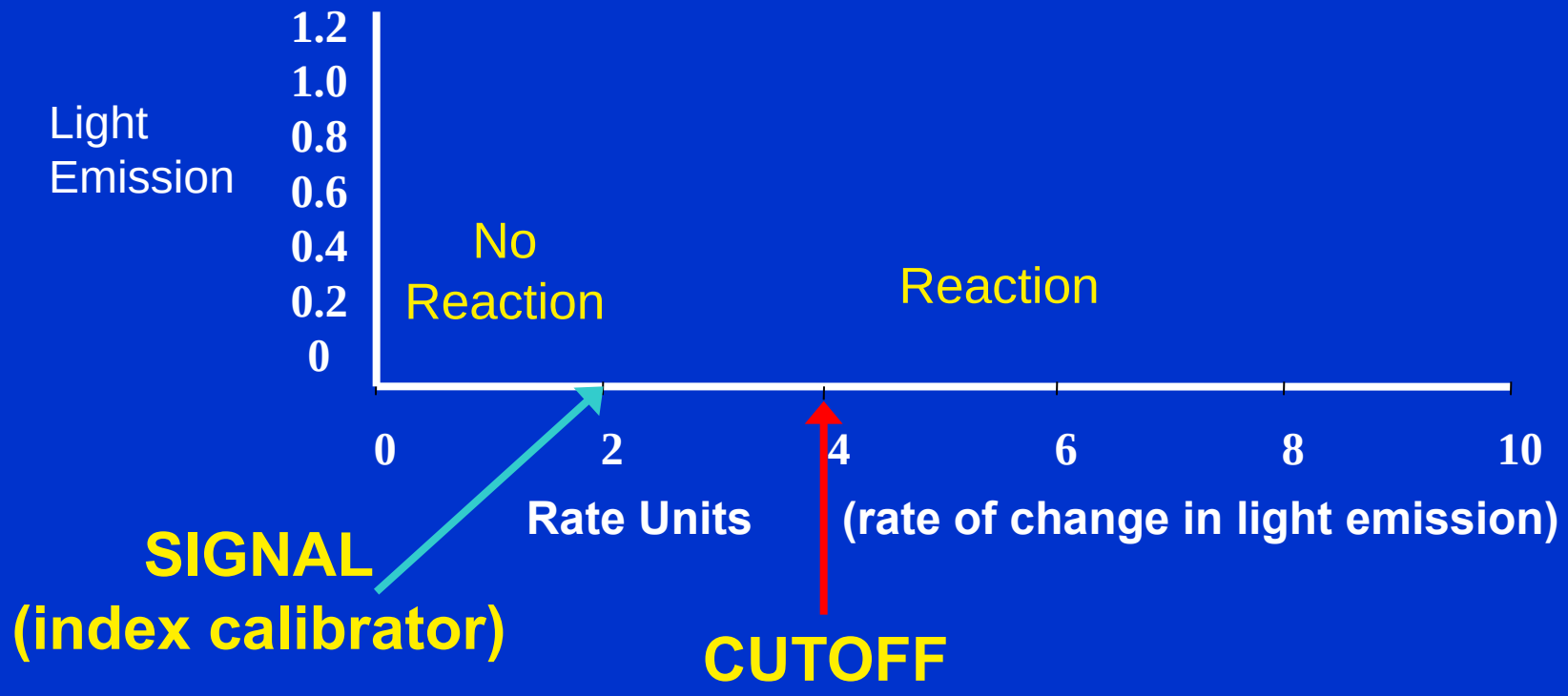
What makes Serology assays different from Biochemical Immunoassays?

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

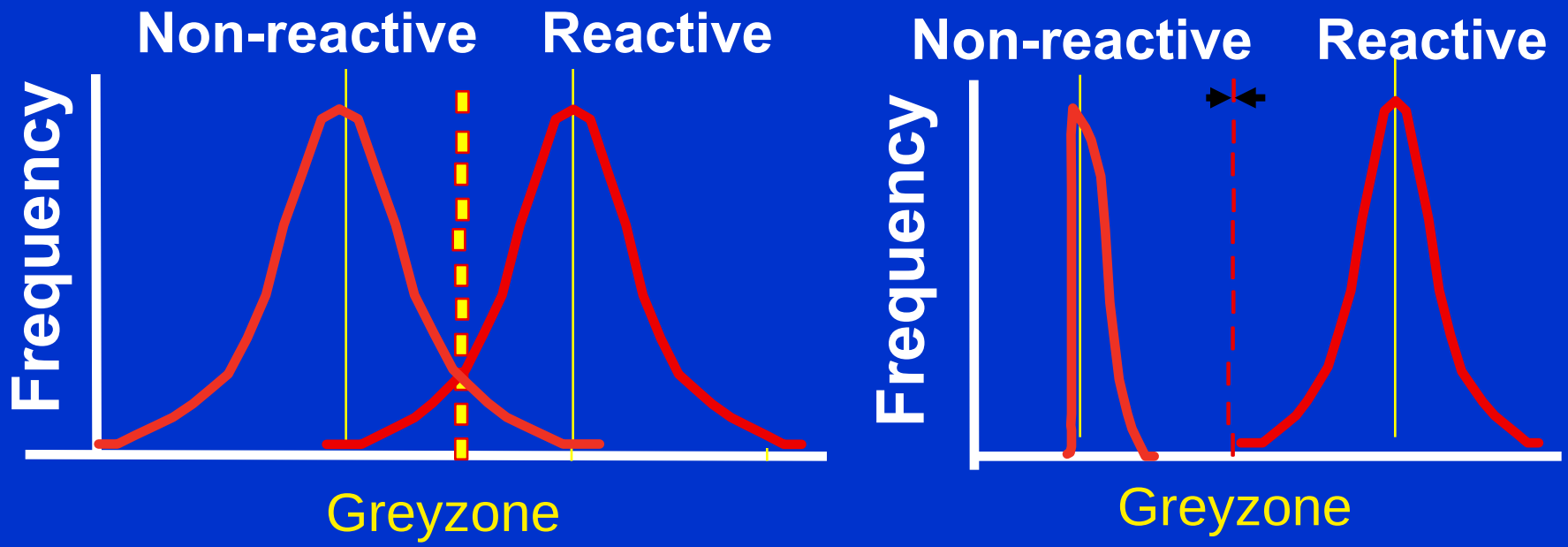
QUALITATIVE v.s. QUANTITATIVE ASSAYS

- **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

GREY ZONE



- 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

Parameter	Description	Formula
Sensitivity	Proportion of TRUE POSITIVES Identified by the Test	**POS Results /** True Positives
Specificity	Proportion of TRUE NEGATIVES Identified by the Test	**NEG Results /** True Negatives

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

- FROM: "Principles and Practice of Clinical Virology" 4th Edition, Edited by Arie J Zuckerman et.al.

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

- Syphillis
- Peptic ulcer disease
- HIV (human immunodeficiency virus)
- Congenital Infections i.e. TORCH
  - **TO**xoplasma, **R**ubella, **C**ytomegalovirus, **H**erpes
- Capsular polysaccharide antigen response
- Viral Hepatitis

PART B: HEPATITIS B

Acute Hepatitis: Differential Diagnosis

= Inflammation of the Liver

TYPE	CAUSE
Viral Hepatitis	Hepatitis A, B, C, D, E CMV, EBV, HSV, VZV, yellow fever
Bacterial Hepatitis	Typhoid fever, Q fever, RMSF, leptospirosis, secondary syphyllis, sepsis
Parasitic Infections	Toxocanasis, liver flukes
Drugs	ASA, acetaminophen, INH, rifampin, oral contraceptives, anti-seizure medications, carbenicillin, sulfonamides
Toxins	Alcohol, carbon tetrachloride
Autoimmune Diseases	Autoimmune hepatitis, SLE

VIRAL HEPATITIS: Clinical Manifestations

Symptoms		Signs	
• Malaise	76-94%	• Jaundice	70-90%
• Anorexia	71-96%	• Hepatomegaly	14-69%
• Dark Urine	65-94%	• Tender liver	20-86%
• Nausea	61-81%	• Rash	40%
• Abdominal pain	26-68%	• Splenomegaly	3-21%
• Scleral icterus	48%	• Fever	1-8%
• Vomiting	20-37%	• High LFT's	100%

Asymptomatic → Symptomatic → Fulminant liver failure → Death

Lab Tests on Line: Viral Hepatitis - 1

http://www.labtestsonline.org.au/

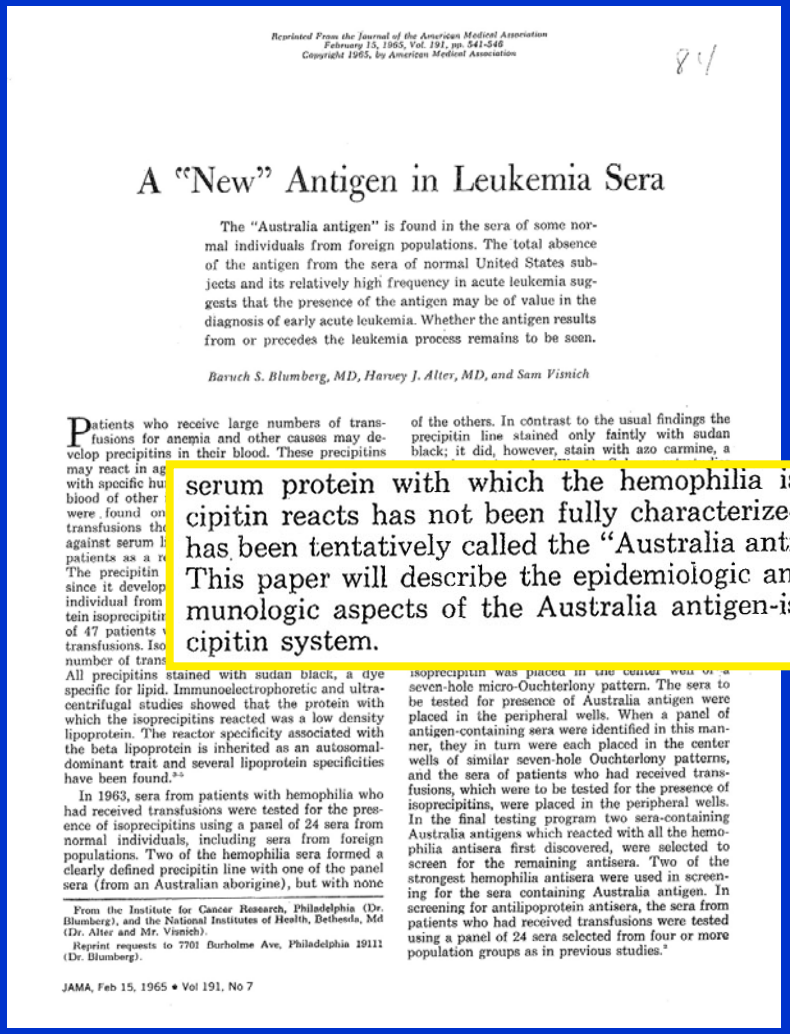
Virus	Hepatitis A	Hepatitis B	Hepatitis C
Transmission route	Faecal - oral	Infected needle or blood, sexual contact	Infected needle or blood, sexual contact
Incubation time (acute infection)	15 – 50 days	45 – 160 days	14 – 180 days
Onset	Sudden	Either sudden or slow, unnoticed	Usually slow unnoticed
Severity	Mild	Occasionally severe	Usually slow developing and symptoms not specific or strong
Chronic Form	No	Yes	Yes
Associated with other diseases	None	Liver cancer, cirrhosis	Liver cancer, cirrhosis

Lab Tests on Line: Viral Hepatitis - 2

Virus	Hepatitis A	Hepatitis B	Hepatitis C
Testing to diagnose acute infection	HAV Ab IgM	HBsAg, Anti-HBc IgM	Anti-HCV, HCV RNA (note may have same results as in chronic hepatitis)
Testing to diagnose chronic infection or to monitor treatment	N/A	HBsAg, HBV DNA, HBeAg, anti HBe	Anti-HCV (once), HCV RNA or viral load, HCV genotype (once)
Tests that detect previous infection	HAV Ab IgG	Anti HBs, Anti HBc total	Anti HCV
Vaccine Available?	Yes	Yes	No
Common Treatment	None	Chronic form - Interferon, entecavir, tenofovir, lamivudine, adefovir	Chronic form interferon, usually with ribavirin

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



Reprinted From the Journal of the American Medical Association
February 15, 1965, Vol. 191, pp. 541-546

8 1

A "New" Antigen in Leukemia Sera

The "Australia antigen" is found in the sera of some normal individuals from foreign populations. The total absence of the antigen from the sera of normal United States subjects and its relatively high frequency in acute leukemia suggests that the presence of the antigen may be of value in the diagnosis of early acute leukemia. Whether the antigen results from or precedes the leukemia process remains to be seen.

Baruch S. Blumberg, MD, Harvey J. Alter, MD, and Sam Visnich

Patients who receive large numbers of transfusions for anemia and other causes may develop precipitins in their blood. These precipitins may react in ag
with specific hu
blood of other
were found on
transfusions th
against serum l
patients as a r
The precipitin
since it develop
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of 47 patients
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number of trans
All precipitins stained with sudan black, a dye specific for lipid. Immunoelectrophoretic and ultracentrifugal studies showed that the protein with which the isoprecipitins reacted was a low density lipoprotein. The reactor specificity associated with the beta lipoprotein is inherited as an autosomal-dominant trait and several lipoprotein specificities have been found.[3-5]

In 1963, sera from patients with hemophilia who had received transfusions were tested for the presence of isoprecipitins using a panel of 24 sera from normal individuals, including sera from foreign populations. Two of the hemophilia sera formed a clearly defined precipitin line with one of the panel sera (from an Australian aborigine), but with none of the others. In contrast to the usual findings the precipitin line stained only faintly with sudan black; it did, however, stain with azo carmine, a

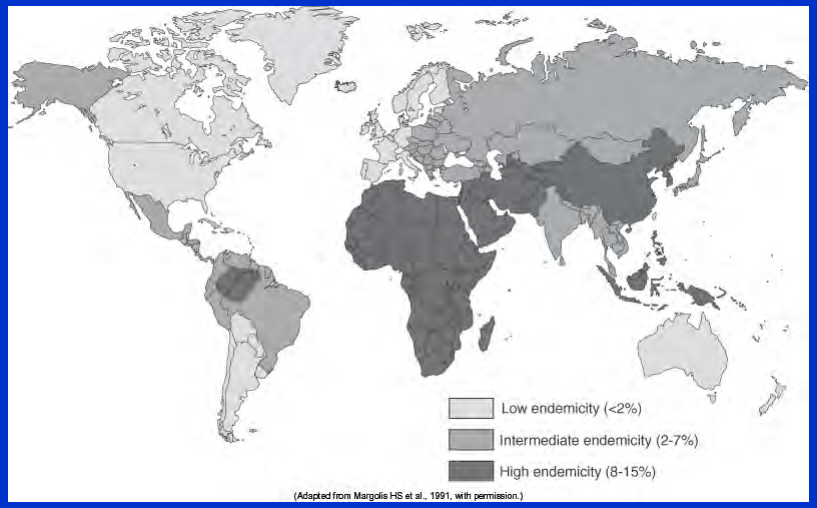
serum protein with which the hemophilia isoprecipitin reacts has not been fully characterized and has been tentatively called the "Australia antigen." This paper will describe the epidemiologic and immunologic aspects of the Australia antigen-isoprecipitin system.

isoprecipitin was placed in the center well of a seven-hole micro-Ouchterlony pattern. The sera to be tested for presence of Australia antigen were placed in the peripheral wells. When a panel of antigen-containing sera were identified in this manner, they in turn were each placed in the center wells of similar seven-hole Ouchterlony patterns, and the sera of patients who had received transfusions, which were to be tested for the presence of isoprecipitins, were placed in the peripheral wells. In the final testing program two sera-containing Australia antigens which reacted with all the hemophilia antisera first discovered, were selected to screen for the remaining antisera. Two of the strongest hemophilia antisera were used in screening for the sera containing Australia antigen. In screening for antilipoprotein antisera, the sera from patients who had received transfusions were tested using a panel of 24 sera selected from four or more population groups as in previous studies.[6]

From the Institute for Cancer Research, Philadelphia (Dr. Blumberg), and the National Institutes of Health, Bethesda, Md (Dr. Alter and Mr. Visnich).
Reprint requests to 7701 Burholme Ave, Philadelphia 19111 (Dr. Blumberg).

Diagram from : http://coursewareobjects.elsevier.com/objects/elr/Wyllie/pediatricGI3e/IC/images/063002.jpg

HEPATITIS B (HBV): WORLD-WIDE

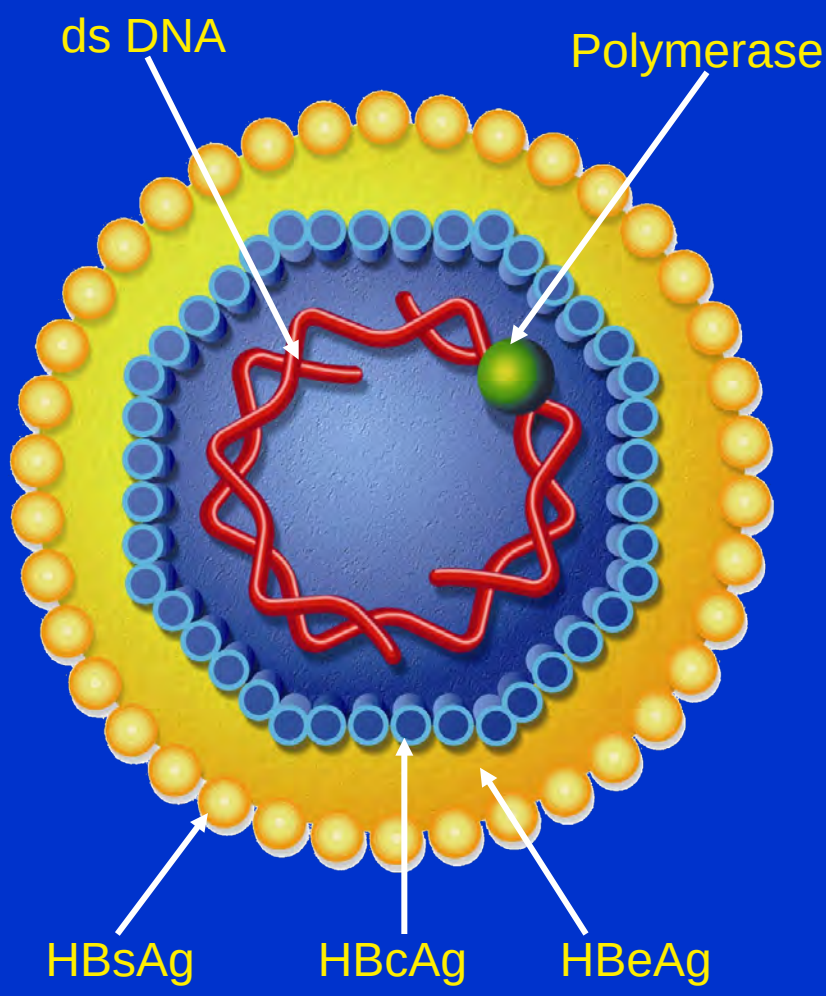


- Estimated 350 million people currently infected
- Worldwide prevalence is about 10-fold higher than the global AIDS cases
- About 80 million chronic carriers have progressed to hepatocellular carcinoma
- > 500,000 deaths occur each year
- Infection can be controlled by universal vaccination, passive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

Diagram from: http://www.cdc.gov/hepatitis/Resources/Professionals/Training/Serology/training.htm

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%



S = surface, c = core, e = early

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

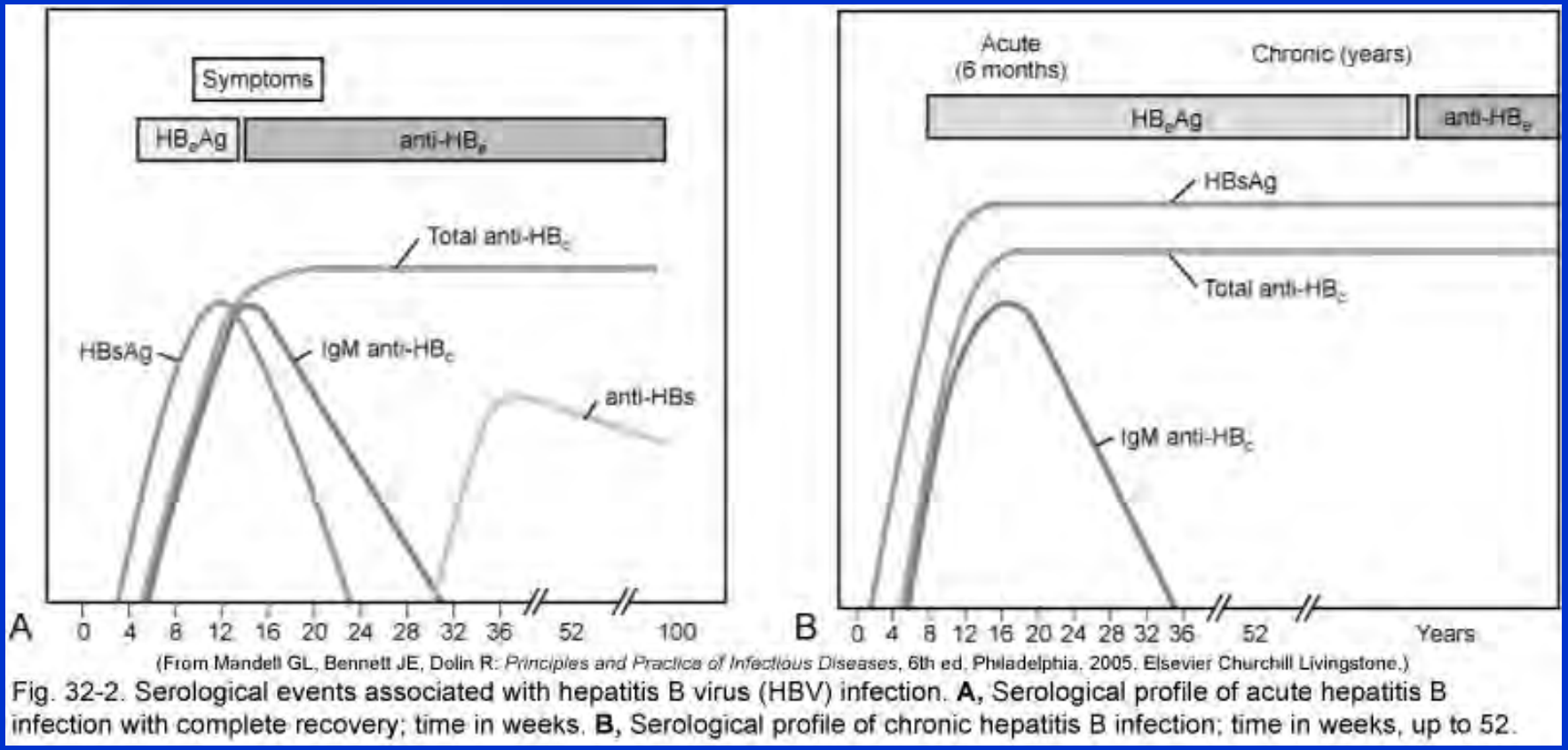
HEPATITIS B: SEROMARKER

Table 1: Description of HBV Seromarkers and Their Diagnostic Outcome	
HBV Seromarker	**HBV infection outcome**
HBsAg	Persistence for longer than 6 months can indicate chronic infection
Anti-HBs	Seroconversion to anti-HBs can indicate immunity
HBeAg	Determines relative infectivity
Anti-HBe	Seroconversion to anti-HBe indicates progression towards infection resolution
Anti-HBc IgM	Differentiates acute/recent infection from chronic carrier state or resolved HBV infection
Anti-HBc	Helps to establish the stage of chronic infection
HBV DNA	Persistence without seromarkers can indicate occult infection

http://evolvels.elsevier.com/section/default.asp?id=1829_global_0001&mode=

TIME COURSE HEPATITIS B

ACUTE v.s. CHRONIC



(From Mandell GL, Bennett JE, Dolin R: *Principles and Practice of Infectious Diseases*, 6th ed, Philadelphia, 2005, Elsevier Churchill Livingstone.)

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.

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

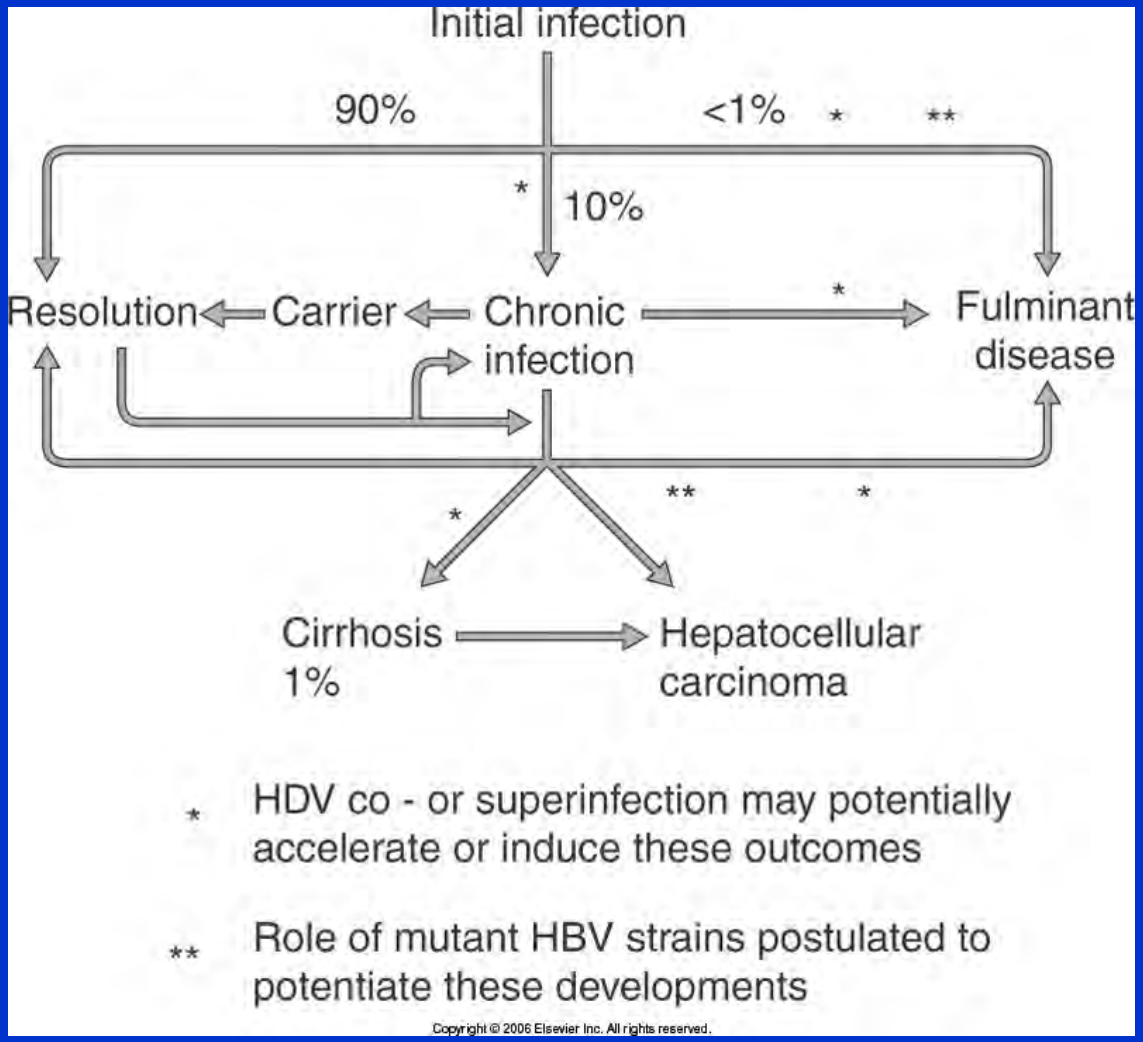
HEPATITIS B: INTERPRETATION

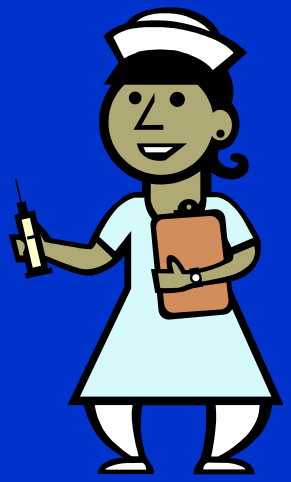
(http://www.cdc.gov/NCIDOD/DISEASES/HEPATITIS/b'faqb.htm)

Table 2: Interpretation of Hepatitis B Serological Test Results		
Tests	**Results**	**Interpretation**
HBsAg anti-HBc anti-HBs	negative negative negative	Susceptible
HBsAg anti-HBc anti-HBs	negative positive positive	Immune due to natural infection
HBsAg anti-HBc anti-HBs	negative negative positive	Immune due to hepatitis B vaccination
HBsAg anti-HBc IgM anti-HBc anti-HBs	positive positive positive negative	Acutely infected
HBsAg anti-HBc IgM anti-HBc anti-HBs	positive positive negative negative	Chronically infected

http://coursewareobjects.elsevier.com/objects/elr/Wyllie/pediatricGI3e/IC/images/063004.jpg

Hepatitis B: Potential Outcomes





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

X

- Option B:
  - She is infected with the hepatitis B virus

X

- Option C:
  - She is not protected from HBV infection

√

ANSWER

- 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

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

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?

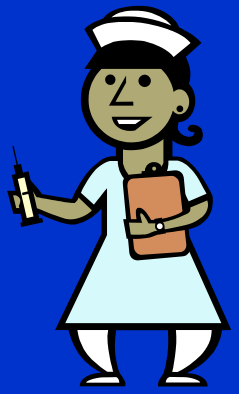
CASE: What needs to be done to protect her from HBV infection?

- Option A:
  - She should have a booster dose of vacine X
- Option B:
  - No postexposure prophylaxis is recommended √
- Option C:
  - She should be tested for hepatitis B surface antigen X

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 X
- Option B:
  - Test only for ALT X
- 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: X
  - 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 X
- Option B:
  - Provide counselling to refrain from blood donation until follow-up testing is done X
- Option C:
  - Provide counselling to followup recommended infection control practices at work X
- 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