

Tourniquet is applied
and area is disinfected



Needle is introduced
into vein, blood is drawn
into vial and analyzed



ADAM.

Lyme, HBV, HCV, WNV Diagnostic Test Interpretation

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Different Serology Technologies

Screening tests

- IFA
- EIA
 - Plate
 - Micro/EIA
 - Color vs chemiluminescent
 - New technologies

Require Acute / conv samples

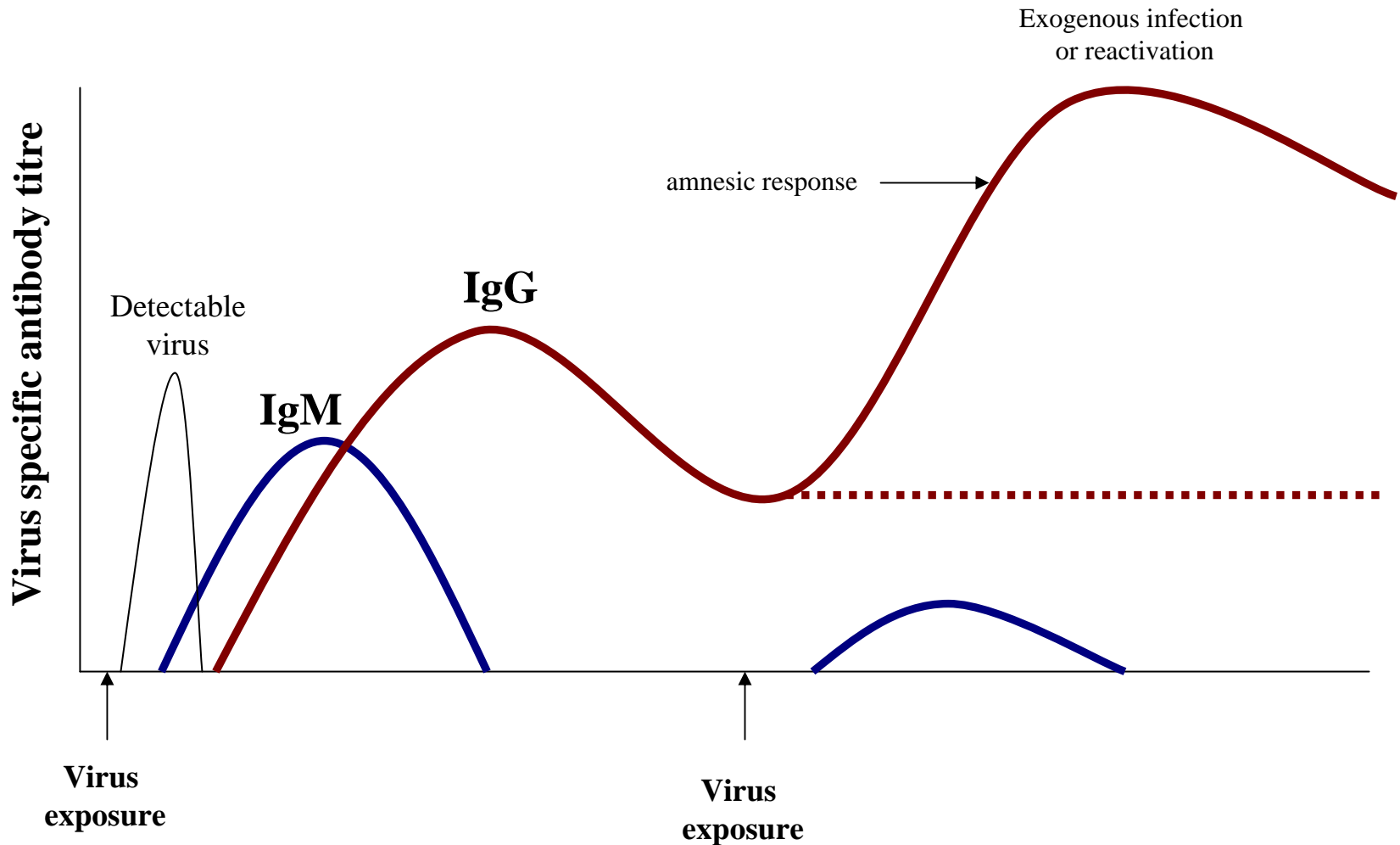
Confirmatory tests

- Avidity testing
- Immunoblot
 - Western
 - recombinant
- Neutralization assays
 - PRNT
 - Microneutralization
- Hemagglutination Inhibition
- Complement fixation

Caveats to Serologic Testing

- Know the typical serologic response
- What is the prevalence of the disease
- What are the performance characteristics of the test
 - No test is 100% sensitive or specific
 - Either a true positive or a false positive
- What potential cross reactions can occur
 - IgM testing often has more false positive results

Typical Humoral Immune response



What does a positive and negative test mean?

Prevalence of HIV	300/1,000,000 (assumes 20% do not know infected)
Individuals affected	300
Individuals not affected	999,700
Kit sensitivity	99.6
Kit specificity	99.9
True positives	298.8
False positives	999.7
True negatives	998700.3
False negatives	1.2
PPV	23%
NPV	99.9999%

Lyme Disease Signs & Symptoms

3 to 30 Days After Deer Tick Bite

- Erythema Migrans
 - Central intensely involved area
 - Most often groin, axilla or thigh
- Fever, chills, headaches
- Muscle and joint pain and fatigue
- 25% do not have the EM lesions



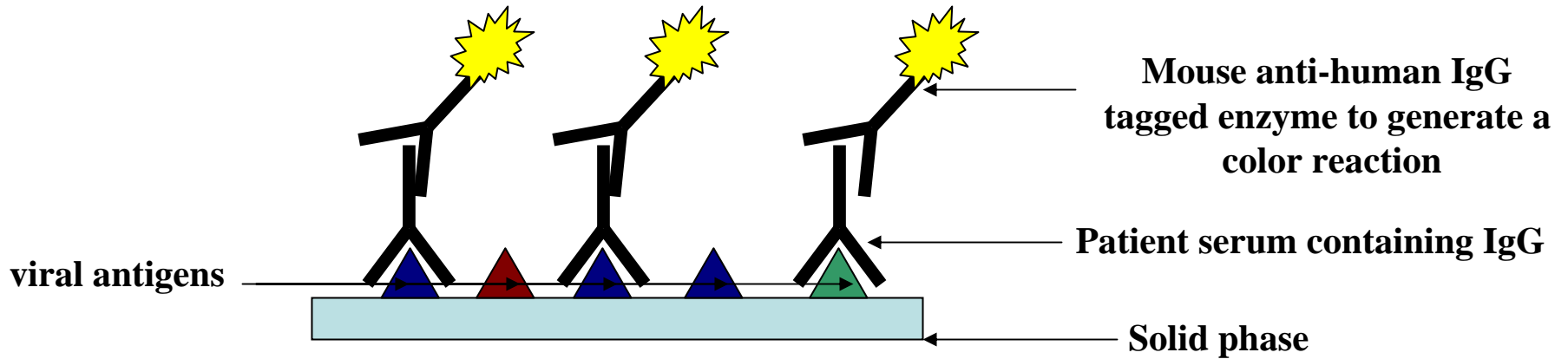
Lyme Disease



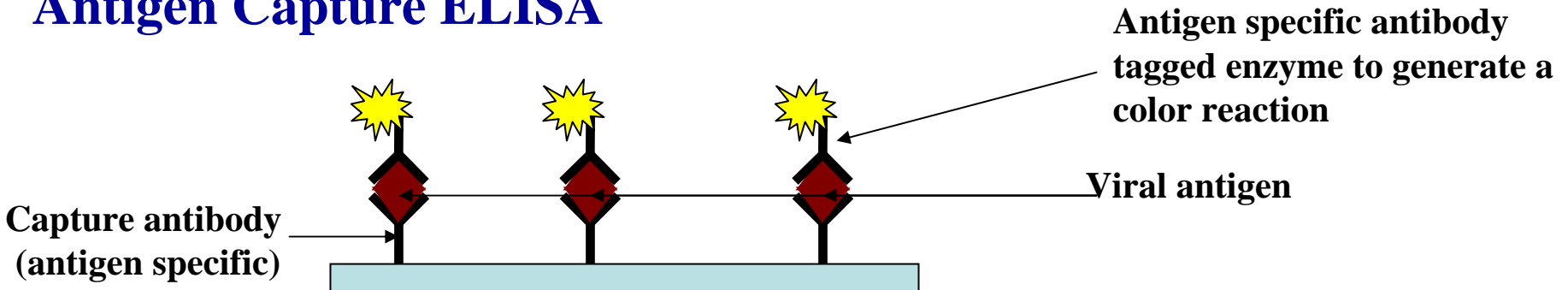
- Serology is complicated
- Diagnosis is a clinical one
- Serology is used to “support diagnosis” not used to make the diagnosis
- CDC / NML recommend a two tiered approach
 - Screen with EIA
 - Confirm screen positives with WB
- Clinical information is very helpful when interpreting and ordering tests

Enzyme-linked Immunosorbent Assay (ELISA)

Antibody ELISA

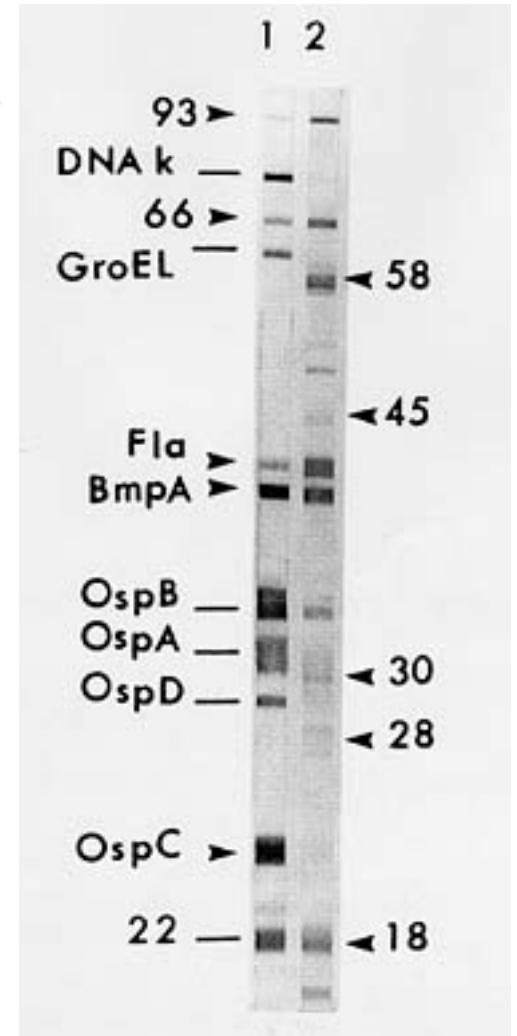


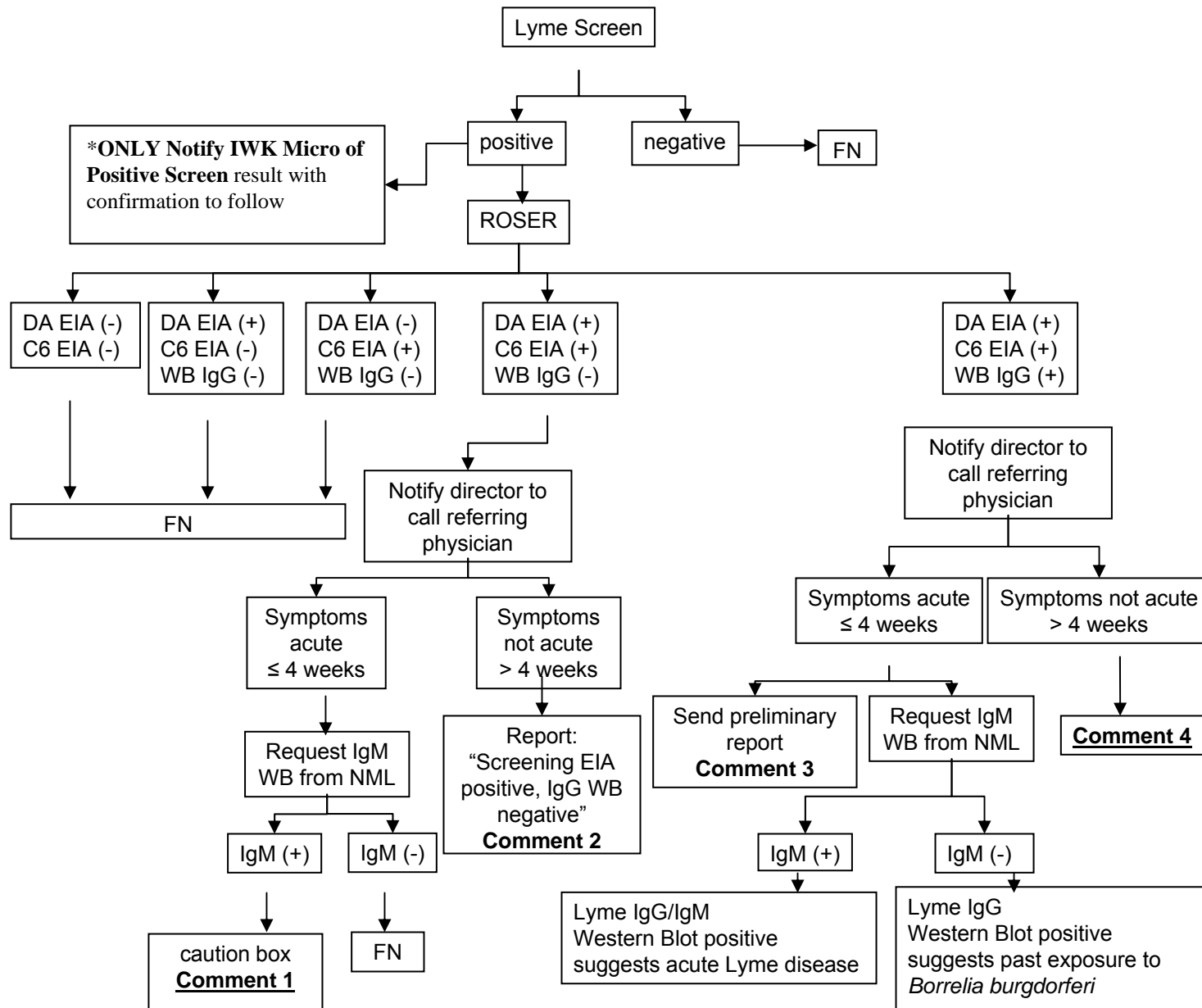
Antigen Capture ELISA



Lyme WB Interpretation

- CDC interpretative criteria
 - IgG WB
 - 5 of 10 bands present
 - IgM WB
 - 2/3 bands present
 - Indeterminate bands are not reported
- IgG WB





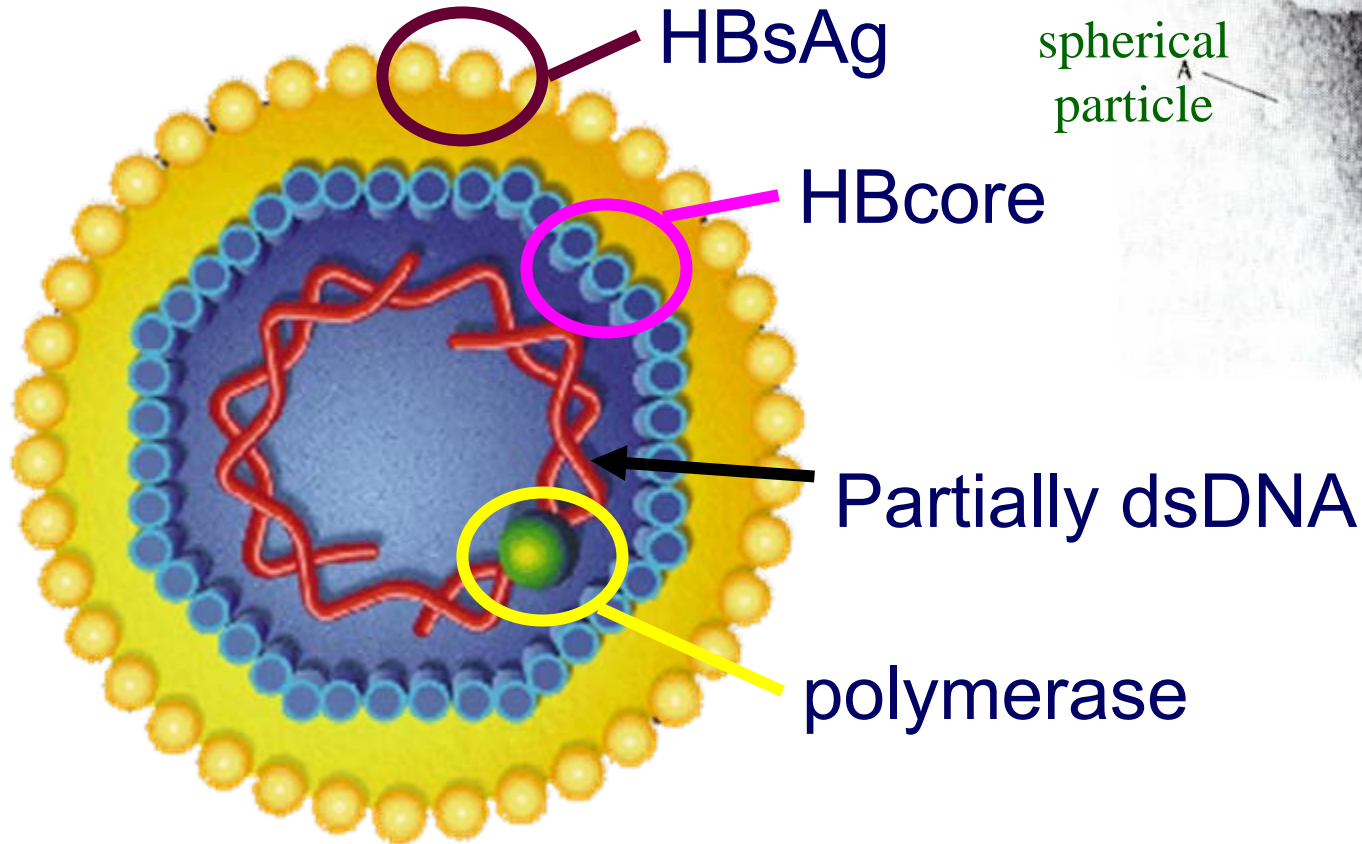
Interpretive Comments for Lyme

- **Comment 1:** Patient has a positive gM Western blot for *Borrelia burgdorferi* but a negative Western blot for IgG. This may reflect either early infection or a false positive result. Early administration of antibiotics may affect the immunologic response to *Borrelia burgdorferi* infection. Suggest repeat serology in one month.
- **Comment 2:** A negative IgG Western Blot in a patient who has been symptomatic for more than 4 weeks suggests the screening EIA results are FALSE POSITIVE reactions.
- **Comment 3:** Preliminary report: Positive IgG Western blot for *Borrelia burgdorferi* IgM Western blot to follow
- **Comment 4:** This patient has been symptomatic for more than 4 weeks. A positive IgG Western Blot suggests past exposure to *Borrelia burgdorferi*. IgM Western blot not performed as it is of limited diagnostic value when used beyond the acute phase of disease.

Caveats to Lyme

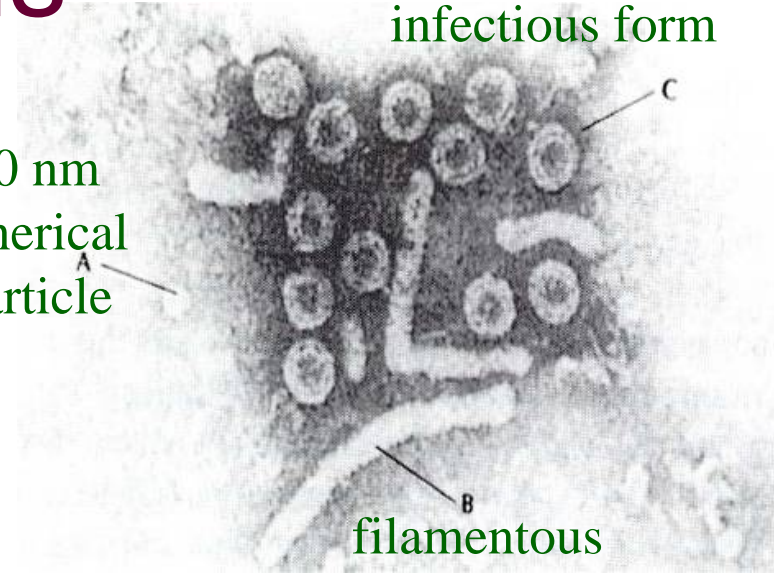
- Presence of typical rash in endemic area does not need serology for diagnosis
- Tests early in the illness may not be “positive” but in an endemic area should not delay Rx in someone with a rash
- Patients with symptoms of greater than 4 weeks duration should have IgG
 - Except if treated early in the course of their illness
- IgM can persist for years
- IgM can be false positive

Hepatitis B Virus



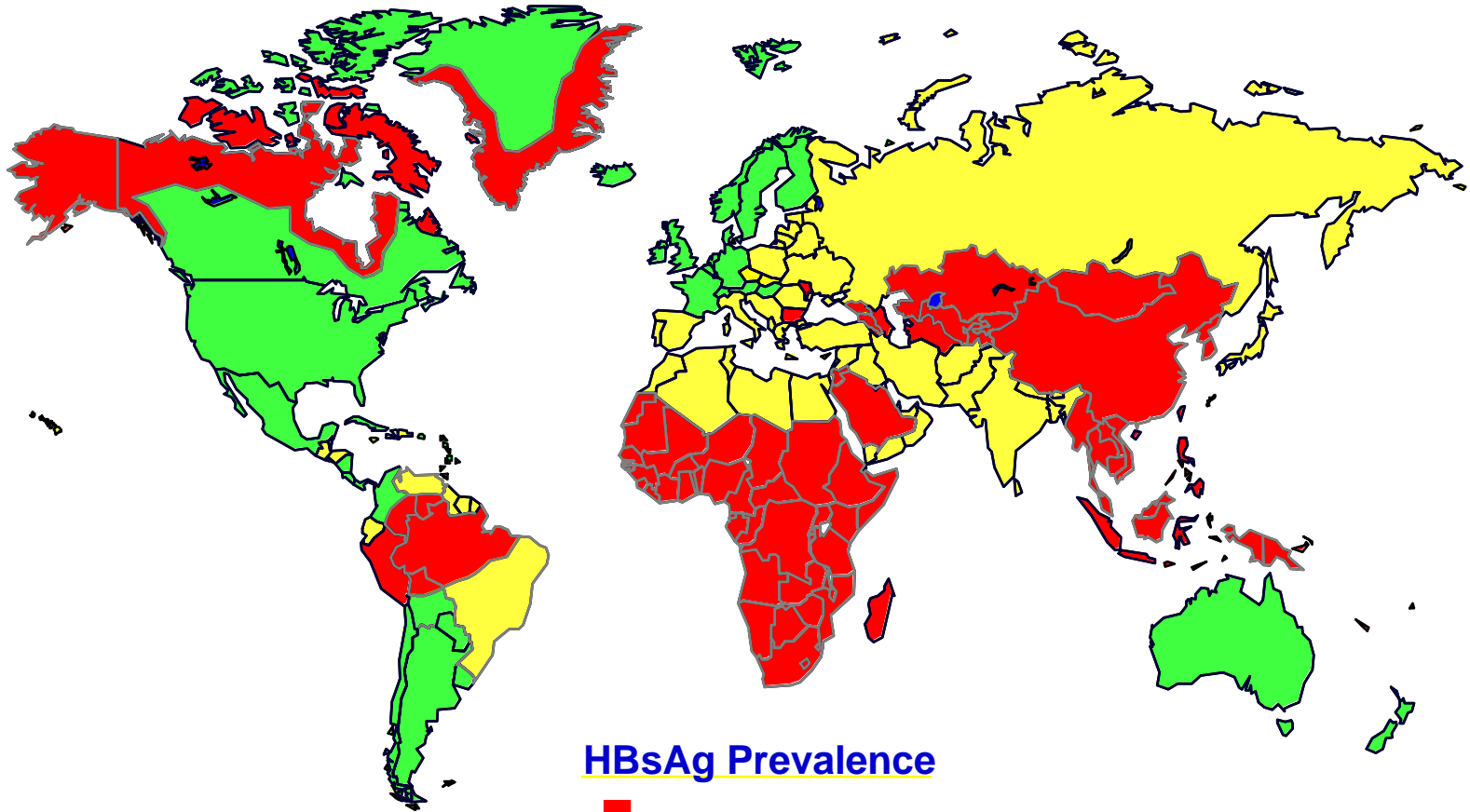
20 nm
spherical
particle

42 nm
Dane particle,
infectious form



filamentous
form

Geographic distribution of chronic HBV infection



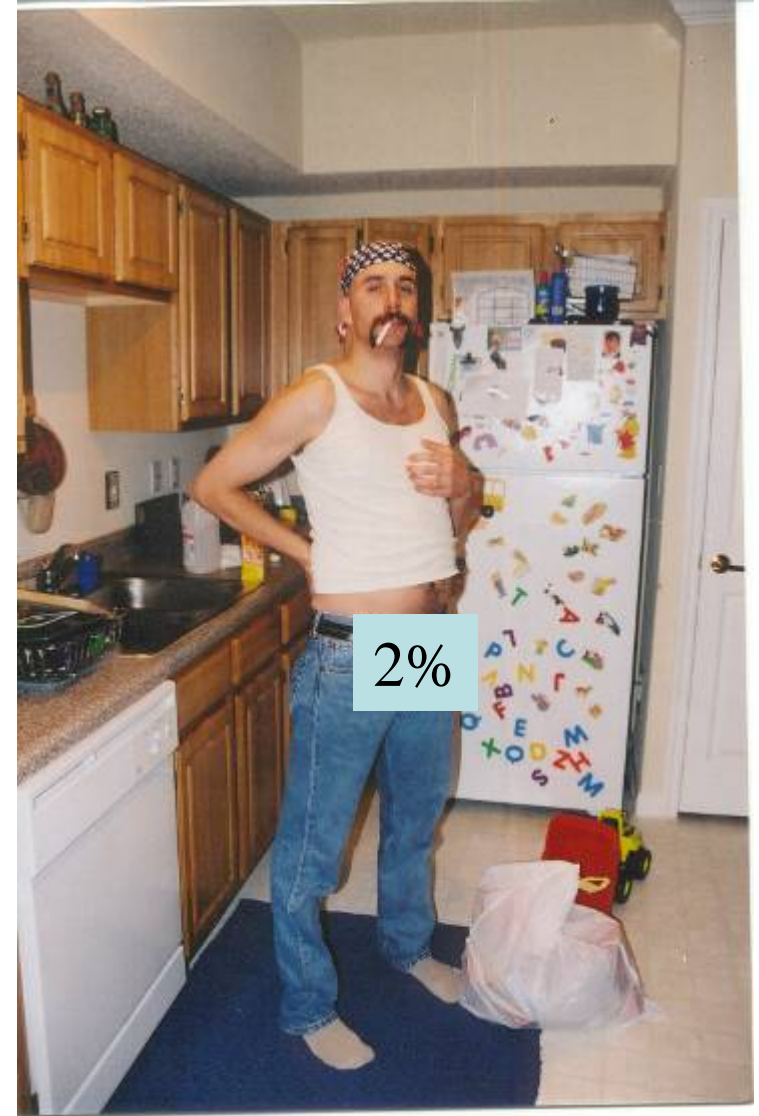
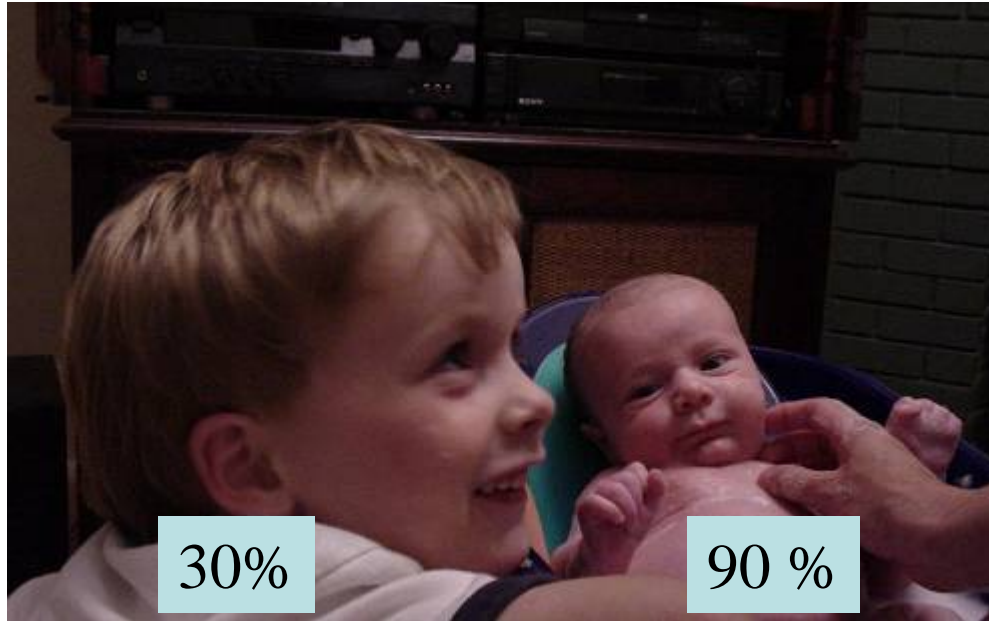
HBsAg Prevalence

- $\geq 8\%$ - High
- 2-7% - Intermediate
- $< 2\%$ - Low

HBV

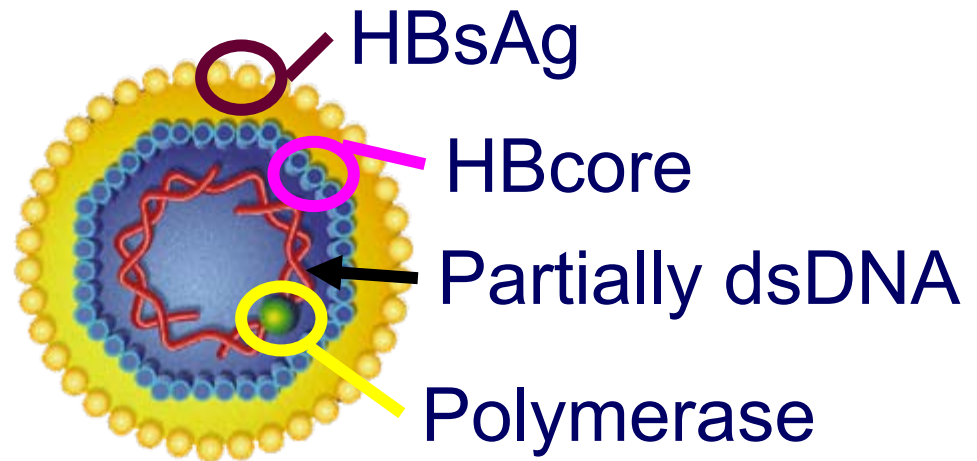
- 1/3 of the world's population (2 billion) have been infected with HBV
 - 3/4 are Asian, high endemicity in sub Sahara Africa (prevalence 10-20% in some regions)
- 400 million people worldwide are chronically infected with HBV
- In the US and Canada, $\approx 0.1-0.3\%$ are carriers.
 - Latest guidelines suggest 600 000 in Canada
 - Individual risk reflects ethnic origin and lifestyle

Natural History Risk of Chronicity



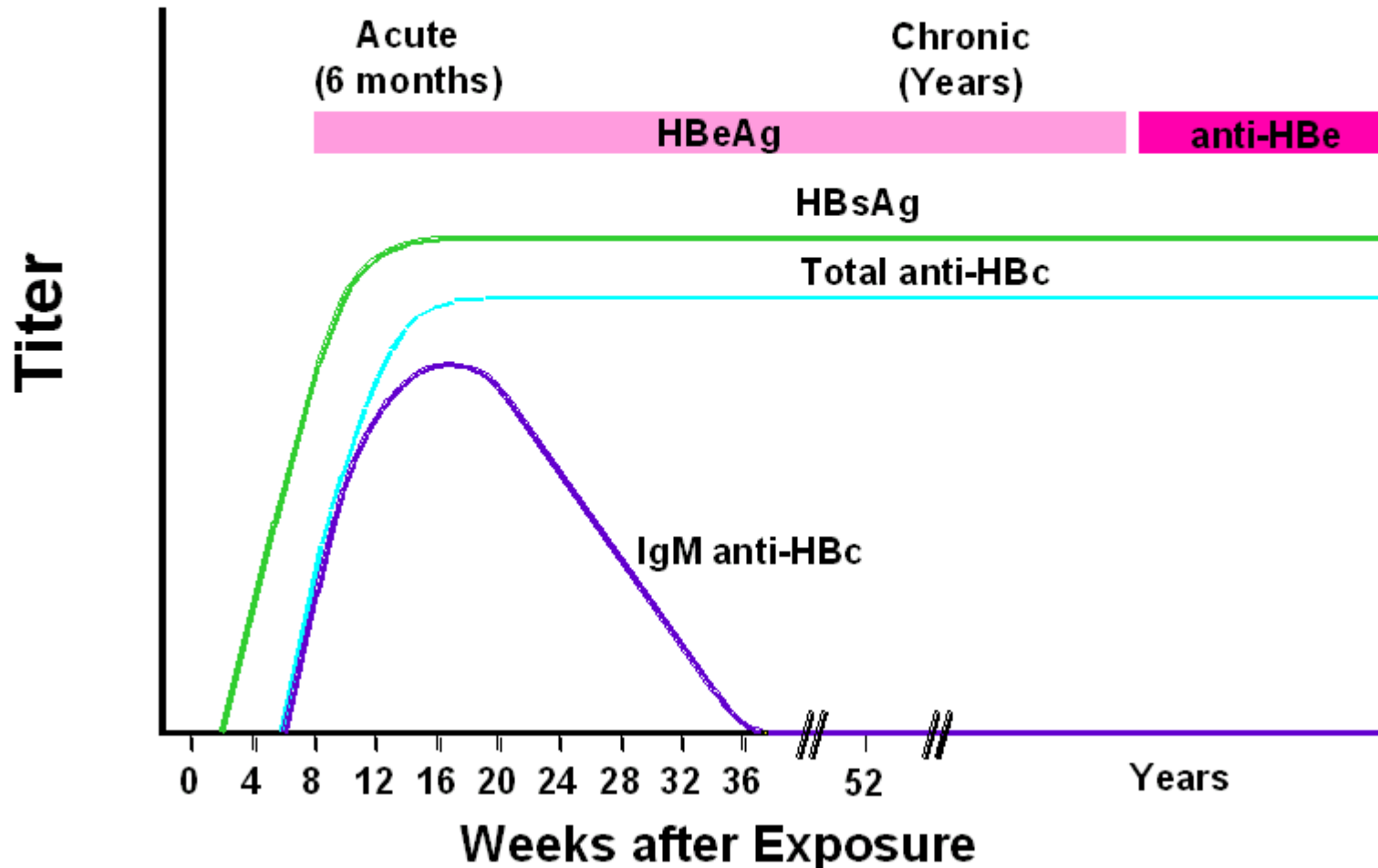
Diagnostic tests

- HBsAg
- Anti-HBsAg Abs
- Anti-HBcAbs
 - Total anti-HBc
 - IgM HBc
- HBeAg / anti-HBeAg Abs
- HBV DNA
 - Viral load helpful in therapeutic decisions



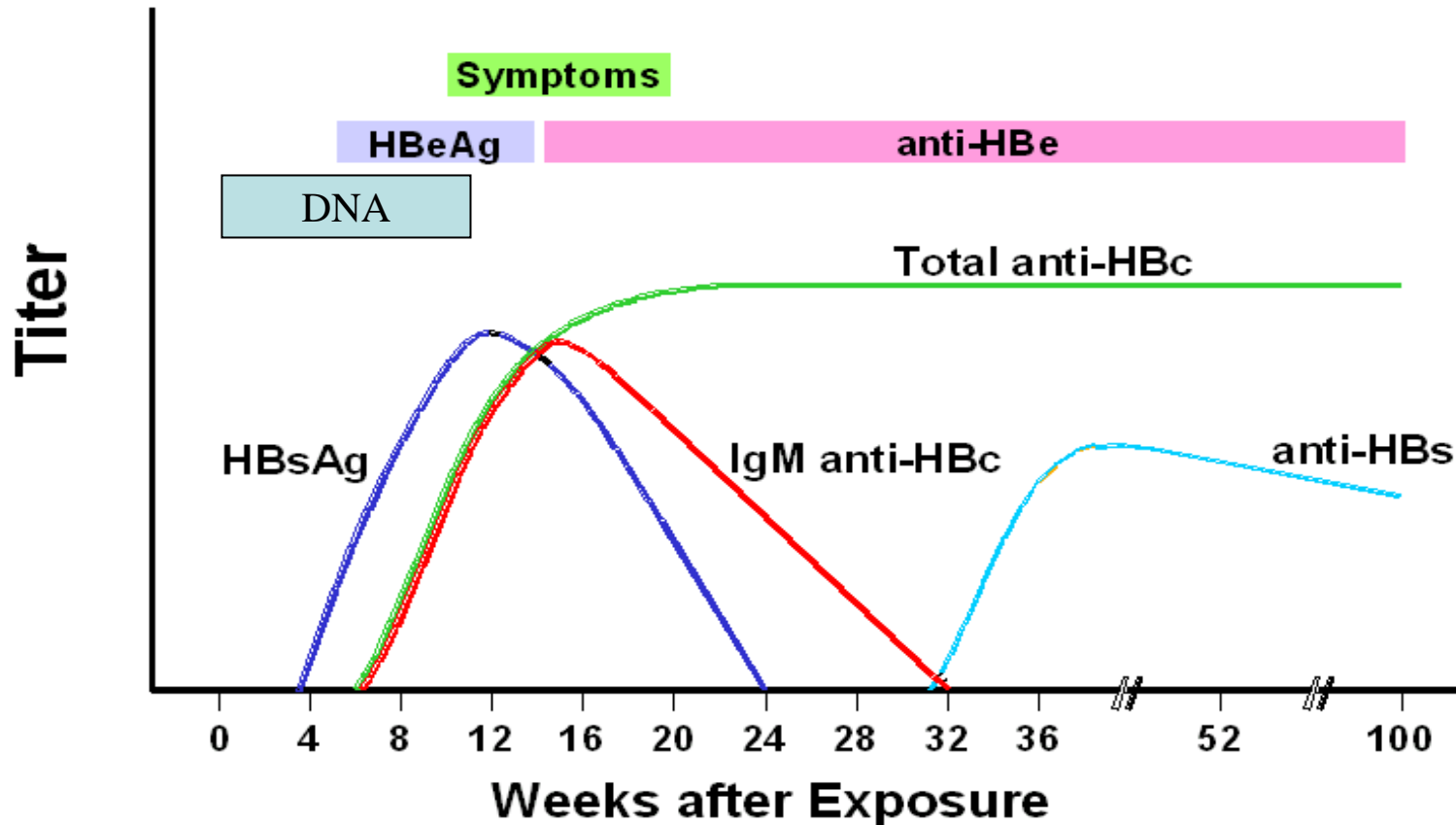
<http://www.cdc.gov/hepatitis/images/pictures/virus.gif>

Progression to Chronic Hepatitis B Virus Infection Typical Serologic Course



Daily production of virions $\sim 10^{11}$ viral particles/day

Acute Hepatitis B Virus Infection with Recovery Typical Serologic Course



- Incubation period:
 - Average 60-90 days (Range 45-180 days)
- DNA peak 127 +/- 47 days – 10^{10} copies/ml (Can produce 10^{13} virions/day)

Diagnosis of Hepatitis B

HBsAg	Anti-HBsAg	Anti-HB core	Interpretation	
Positive	Negative	Positive	Chronic infection*	
Negative	Positive (>10 IU)	Negative	Vaccine induced anti-bodies	Protected
Negative	Positive (>10 UI)	Positive	Previous natural infection and seroconversion	Protected from future infection
Negative	Positive (< 10 IU)	Negative	Vaccine induced antibodies	Possible vaccine failure Repeat vaccine series

* must be HBsAg positive on two occasions at least 6 months apart

Diagnosis of Hepatitis B

- HBeAg/anti-HBe
 - Used to assess how active the infection is
 - Can be used to sort out
 - HBsAg indeterminates
 - “isolated core”
 - Seroconversion to anti-HBe a goal of therapy
- HBV DNA (viral load)
 - Helps with monitoring therapy
 - Used to assess risk of infection
 - 800IU is the lowest VL associated with transmission

HBV infection

Chronic HBV

Active

Inactive
"healthy carriers"

Resolved

- HBsAG + > 60 mo.
- DNA > 20,000 copies/ml
- Persistent or intermittent ↑ of LFTs
- Liver Bx showing chronic hepatitis

- HBsAG + > 60 mo.
- HbeAg - ; anti-HBeAg Abs +
- DNA < 2000 IU/ml
- Persistent normal LFTs
- Liver Bx showing absence of significant hepatitis (necroinflammatory score <4)

- HBsAG negative
- Undetectable DNA
- Persistent normal LFTs

HbeAg +

HbeAg -
"precore mutants"

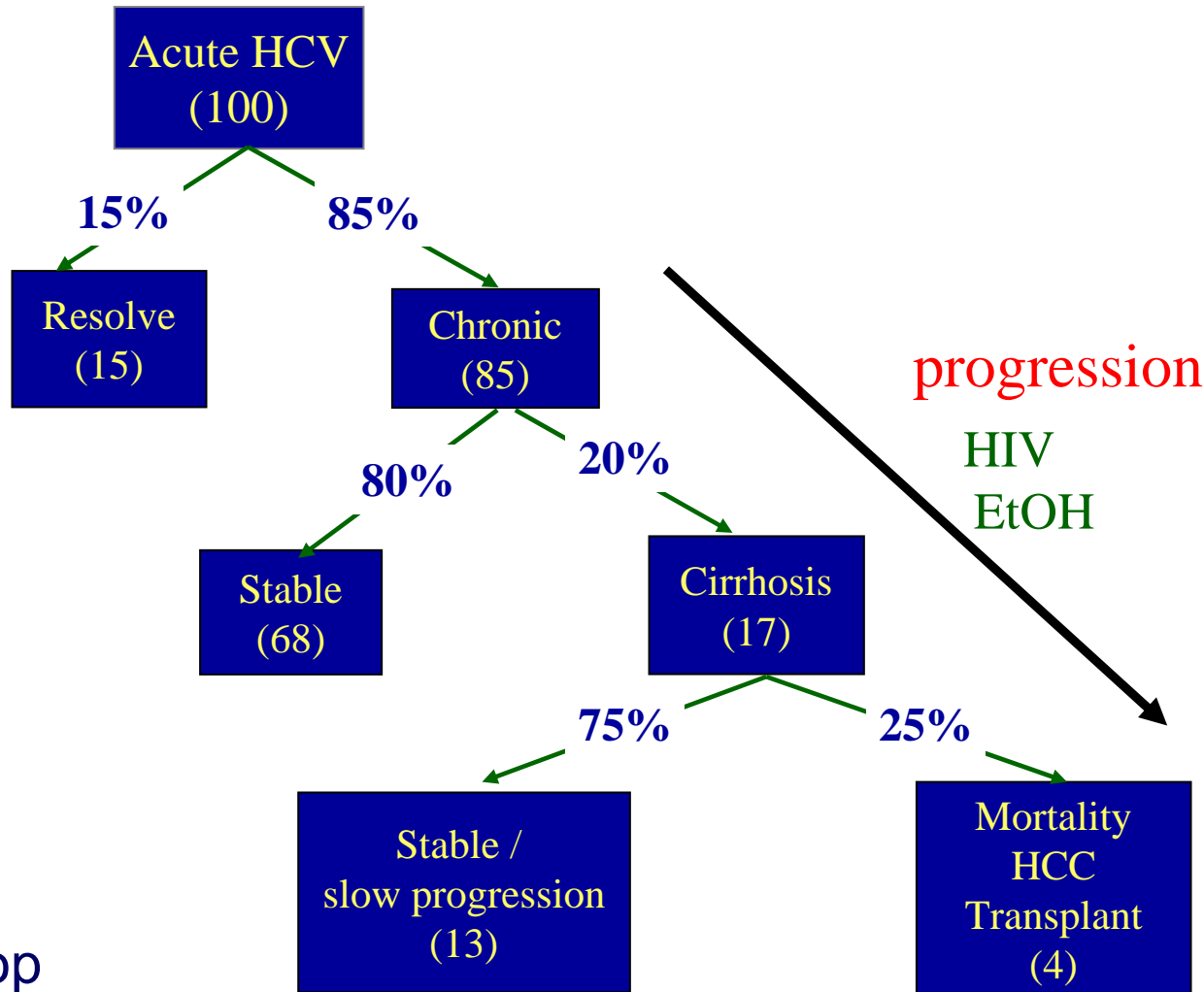
Natural History of HCV

Acute disease

- Incubation period 6-7 weeks (range 2-26 wk)
- Clinical signs in 30-40%
- Jaundice in 20-30%

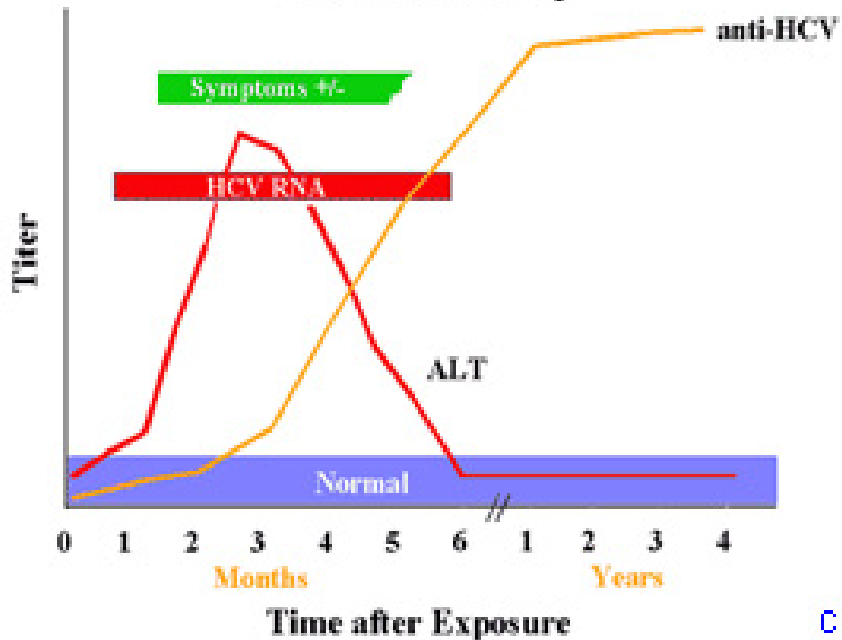
Chronic Disease

- 40-60% may get chronic liver disease (Note higher than for HBV)
- 20% may develop cirrhosis.
- Hepatoma may develop as a long term sequelae.

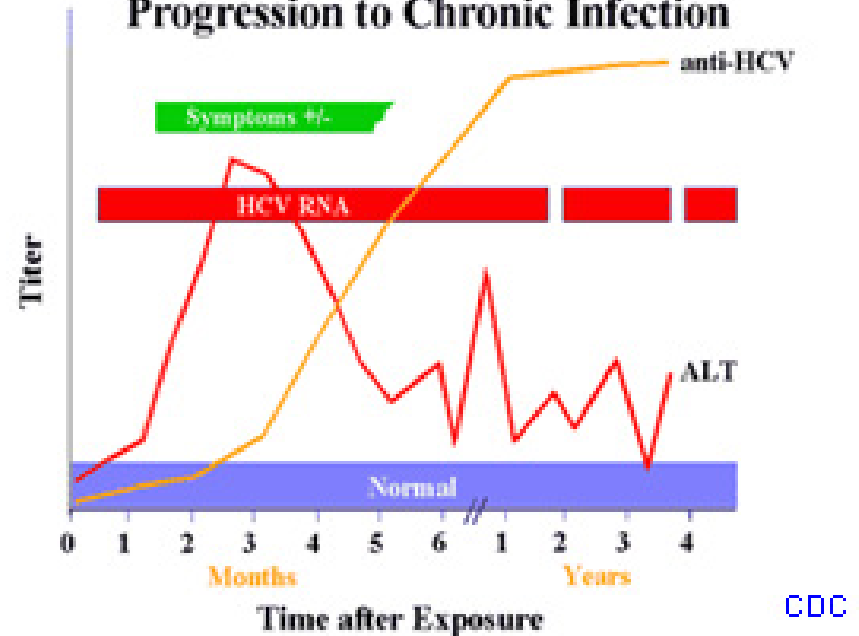


HCV Infection: Typical Serological Course

Serologic Pattern of Acute HCV Infection with Recovery



Serologic Pattern of Acute HCV Infection with Progression to Chronic Infection



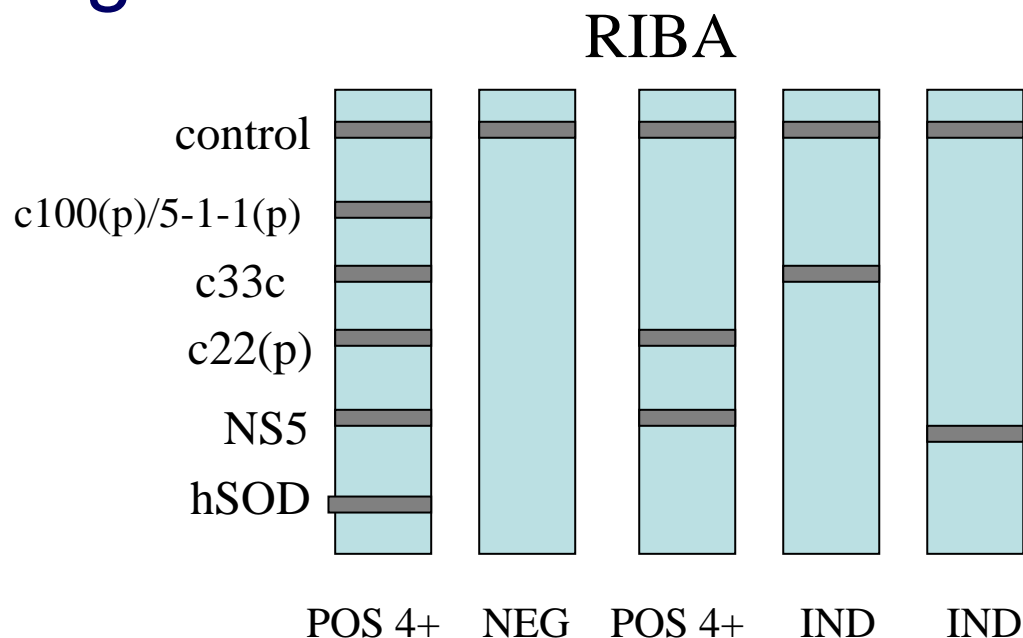
Diagnosis of HCV

- Screening - Antibody Test (EIA)
- Confirmation testing

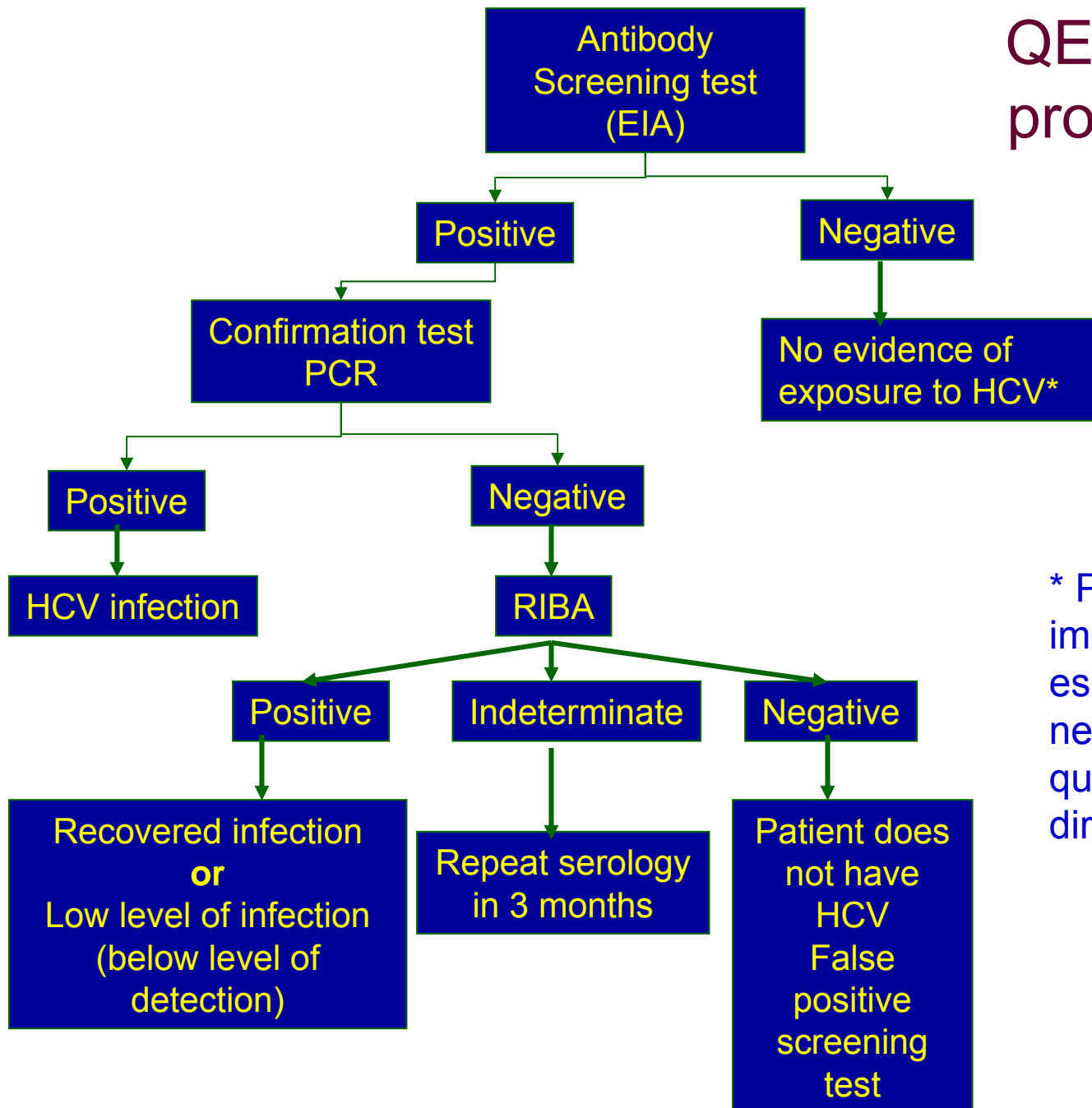
- Qualitative PCR

- Detects viral RNA

- Recombinant Immunoblot Assay (RIBA)



QEI protocol for processing HCV screening



* Patients with immunosuppression esp. HIV may be Ab negative and require qualitative PCR directly

WNIV

Flaviviridae

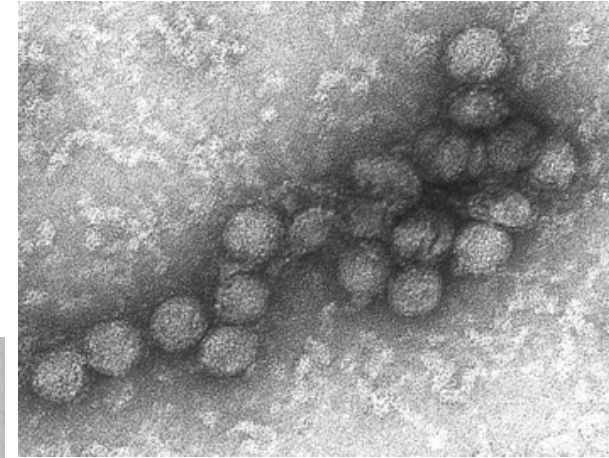
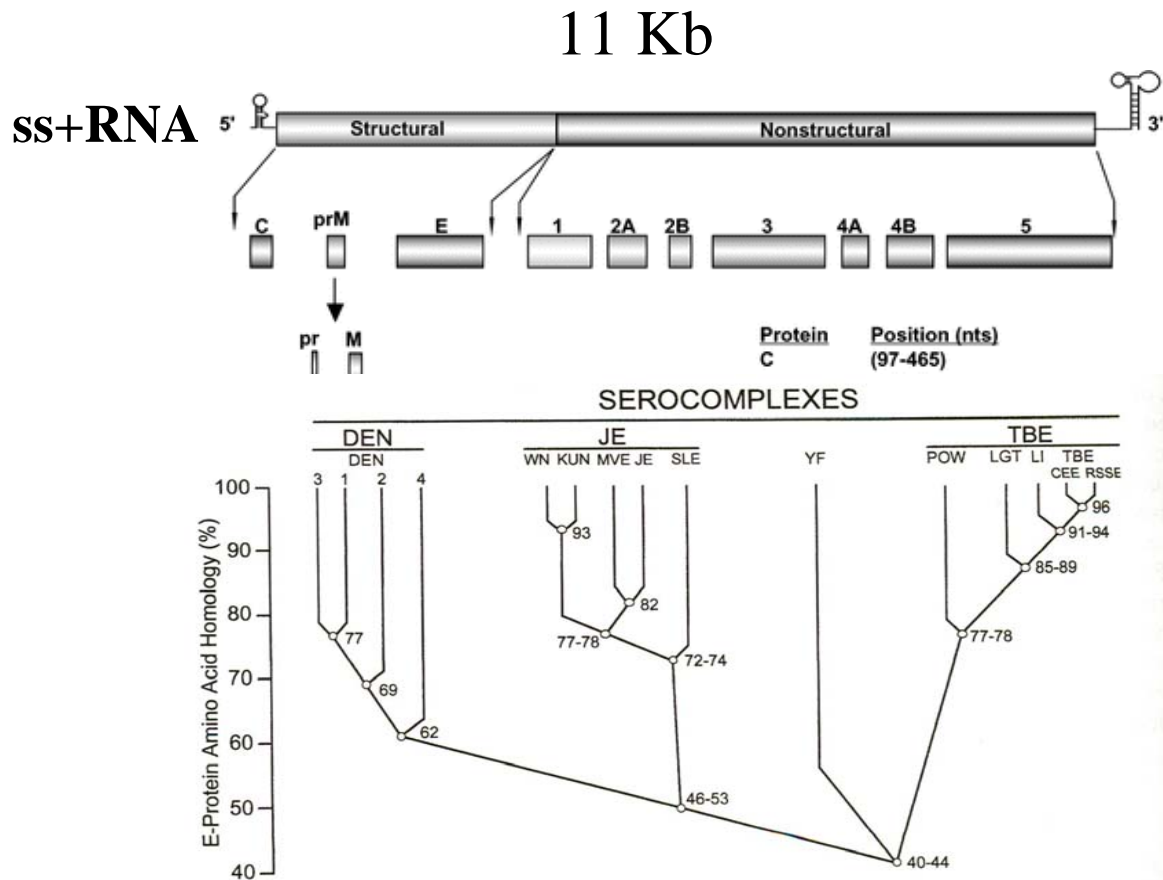
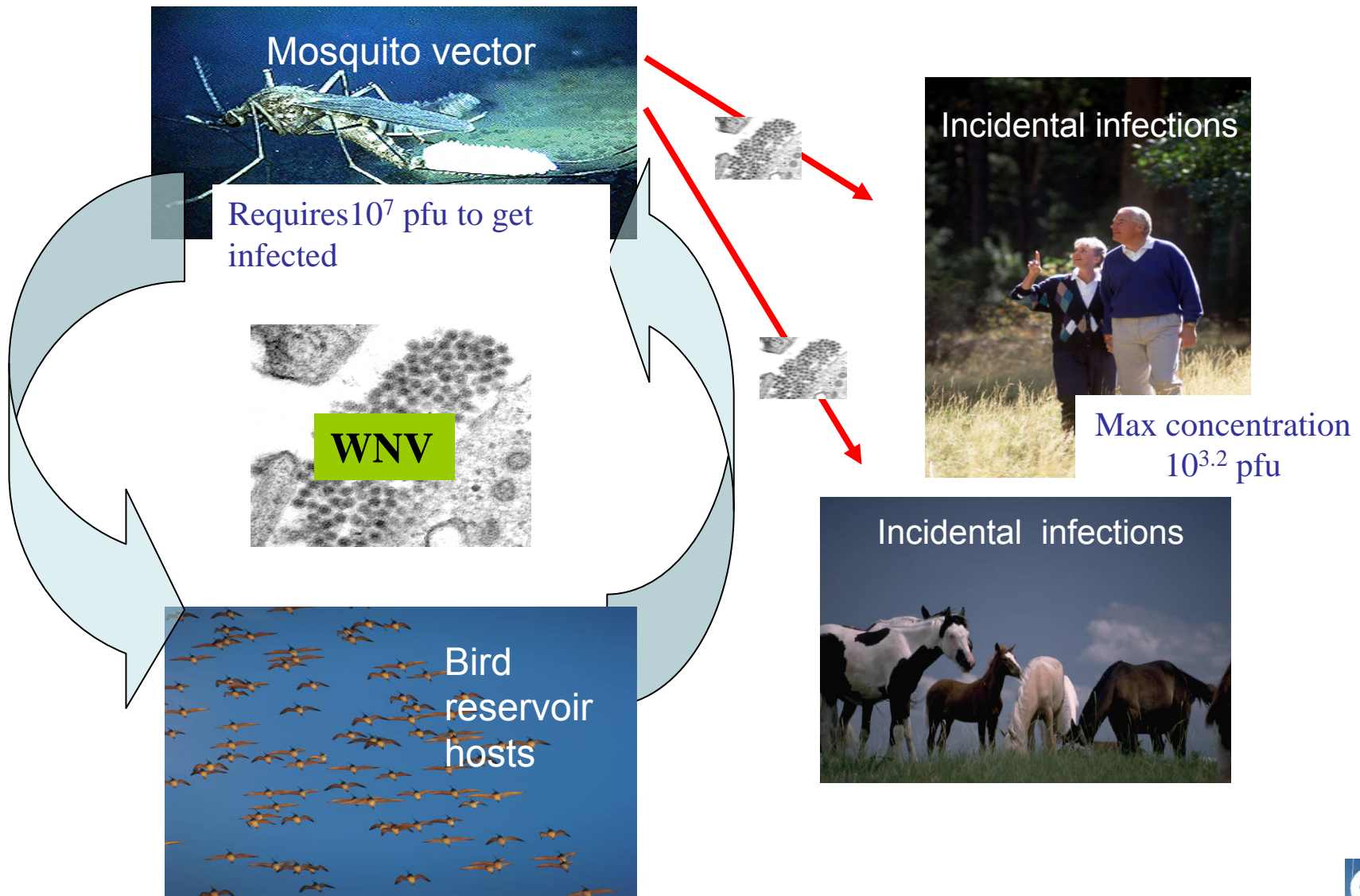
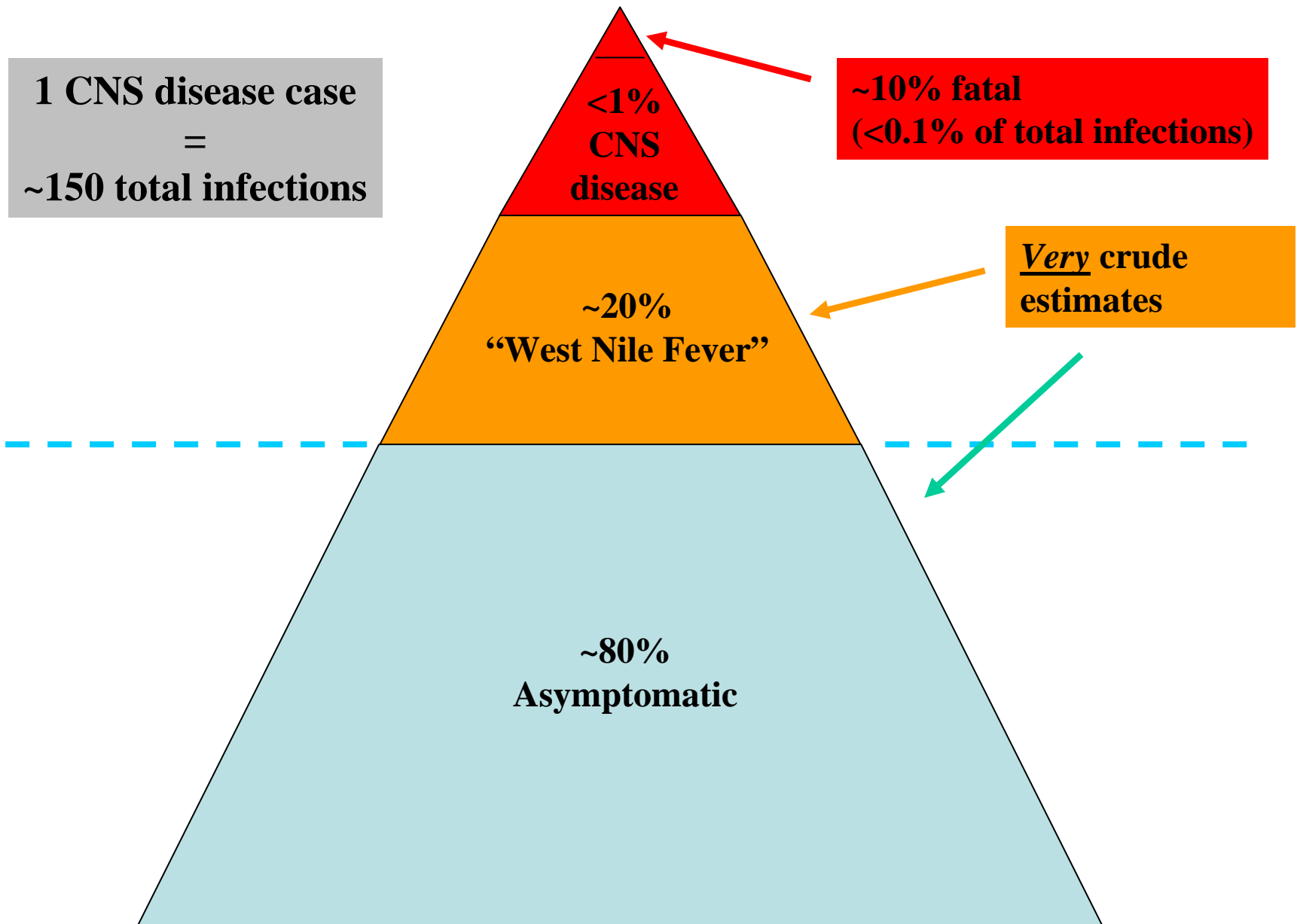


FIG. 2. Evolutionary tree of flaviviruses drawn on the basis of their envelope (E) protein amino acid homologies. These were calculated as percentage identical residues after optimized alignment of compared sequences counting gaps as mismatches using the Beckman Microgenie software package, Version 4.0. DEN, dengue; WN, West Nile; KUN, Kunjin; MVE, Murray Valley encephalitis; JE, Japanese encephalitis; SLE, St. Louis encephalitis; YF, yellow fever; POW, Powassan; LGT, Langat; LI, louping ill; TBE, tick-borne encephalitis. Modified from Mandl et al. (342) and Heinz et al. (221).

West Nile Virus Transmission Cycle

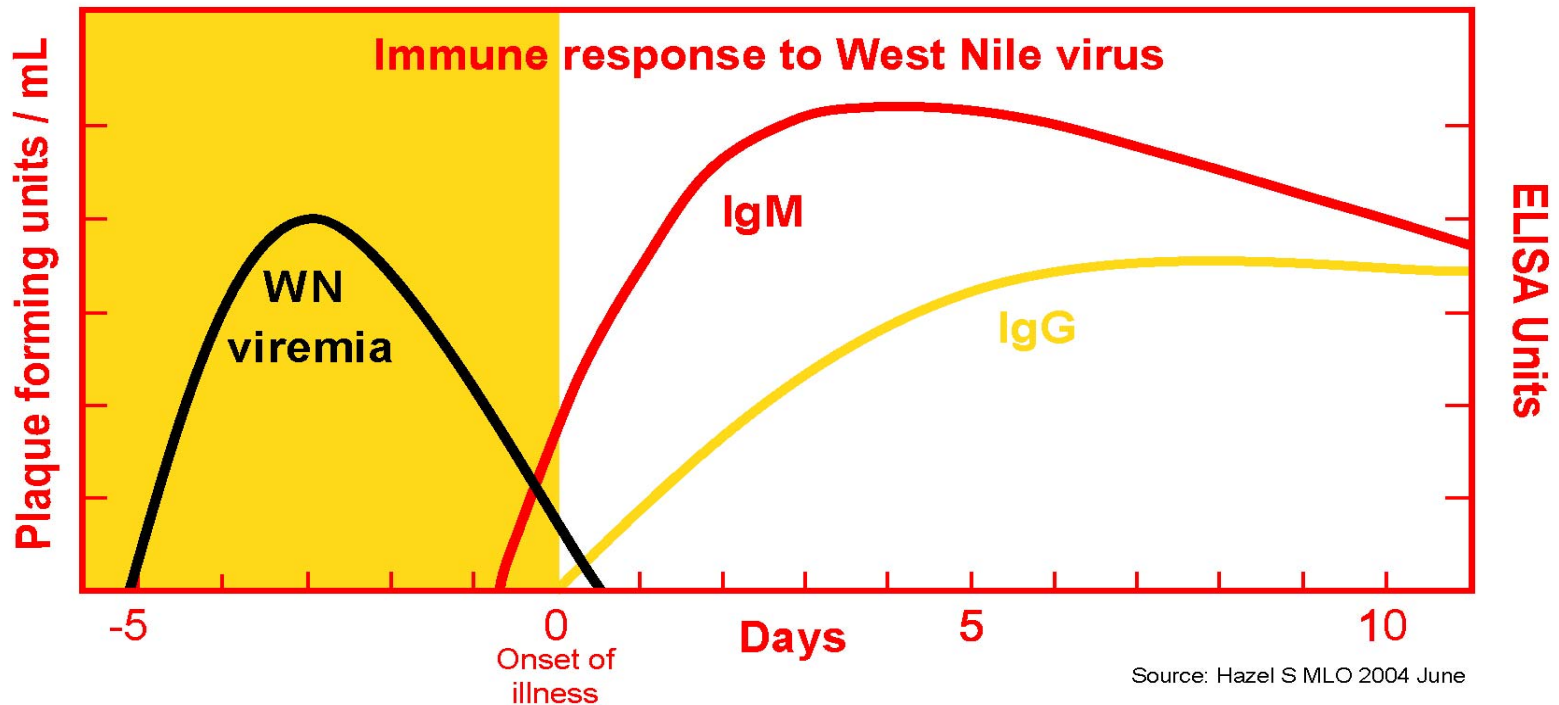


WNV Human Infection “Iceberg”



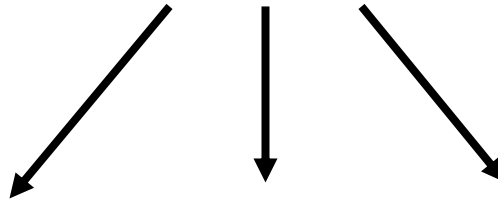
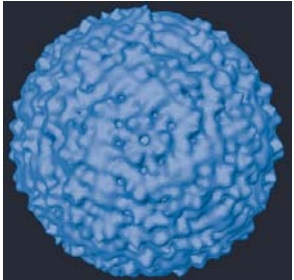
WNV

typical serologic response



<http://www.panbio.com.au/spaw/images/graph.jpg>

WNV DIAGNOSTICS



Isolation/Culture

(Vero Cells, Mice)

Serology

(ELISA, HI, PRNT)

NATs /Ag Detection

(PCR-NASBA / IHC-IFA)

- Serological screening test
 - An IgM Capture EIA test, exact sensitivity will depend on the duration of symptoms and available test

Serological Testing Algorithm for West Nile Virus

Human serum/csf

National Case Definition

Confirmed:

IgM pos csf

IgM pos serum + PRNT

>4-fold increase PRNT titer

IgM ELISA WN & SLE

POS

NEG

Plaque reduction

Neutralization test (PRNT) with:
SLE, WN, (other flaviviruses)

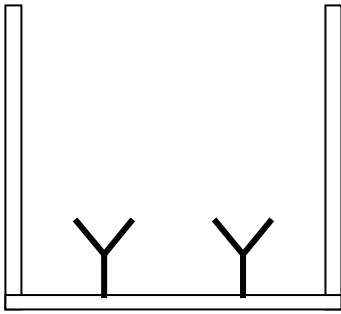
STOP

What does a positive EIA mean?

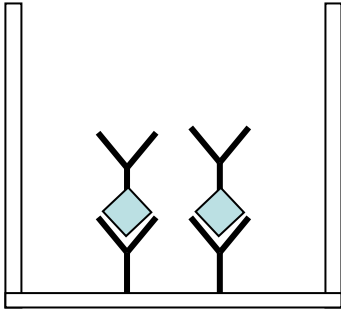
- Is this a true positive or a false positive?
- What is the clinical context
 - When were they tested
 - Why were they tested
 - Travel history
 - Is WNV circulating?
- Need confirmation testing

IgM Capture ELISA

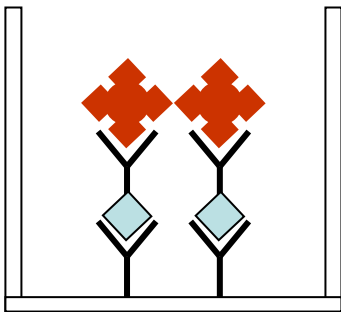
1. Coat With Goat anti-Human IgM



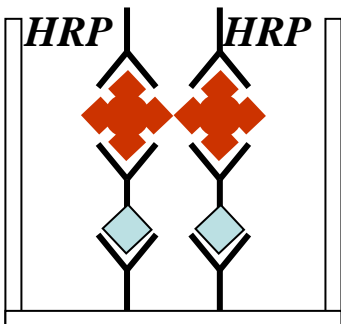
2. Add Patient Serum



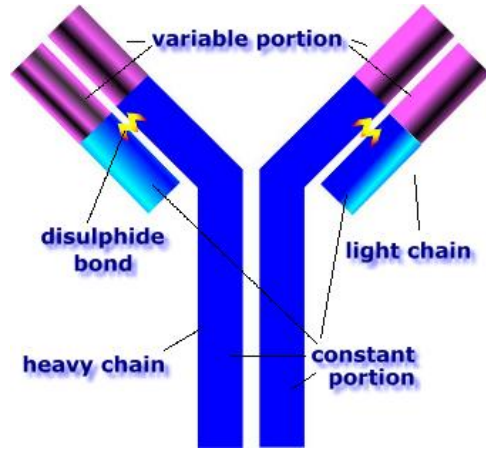
3. Add West Nile Recombinant Antigen



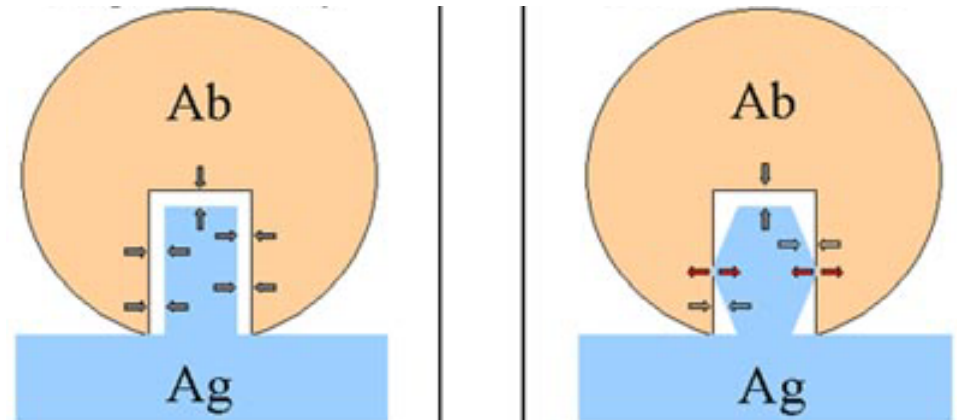
4. Add HRP anti-Flavivirus McAb



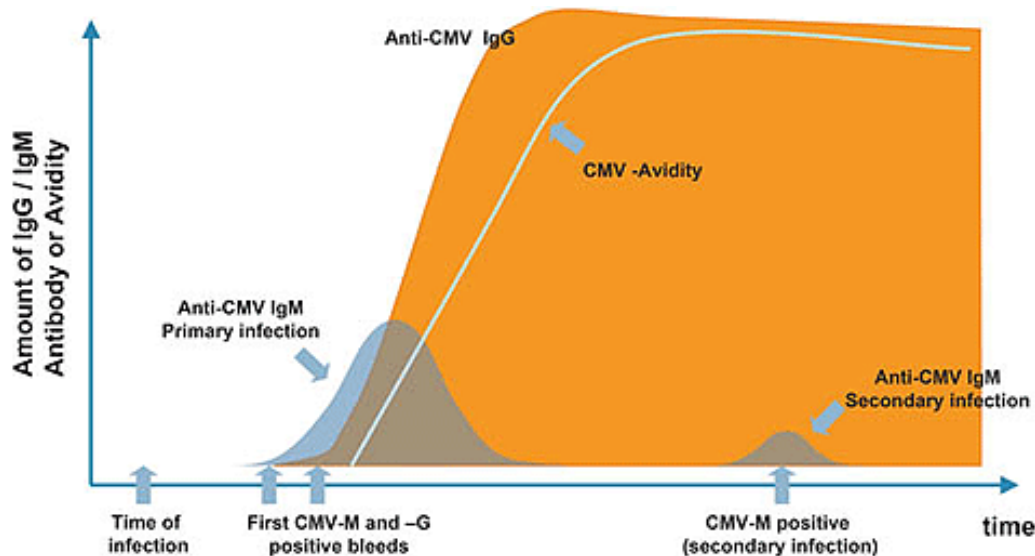
Avidity testing



<http://science.kukuchew.com/wp-content/uploads/2008/04/antibody.jpg>

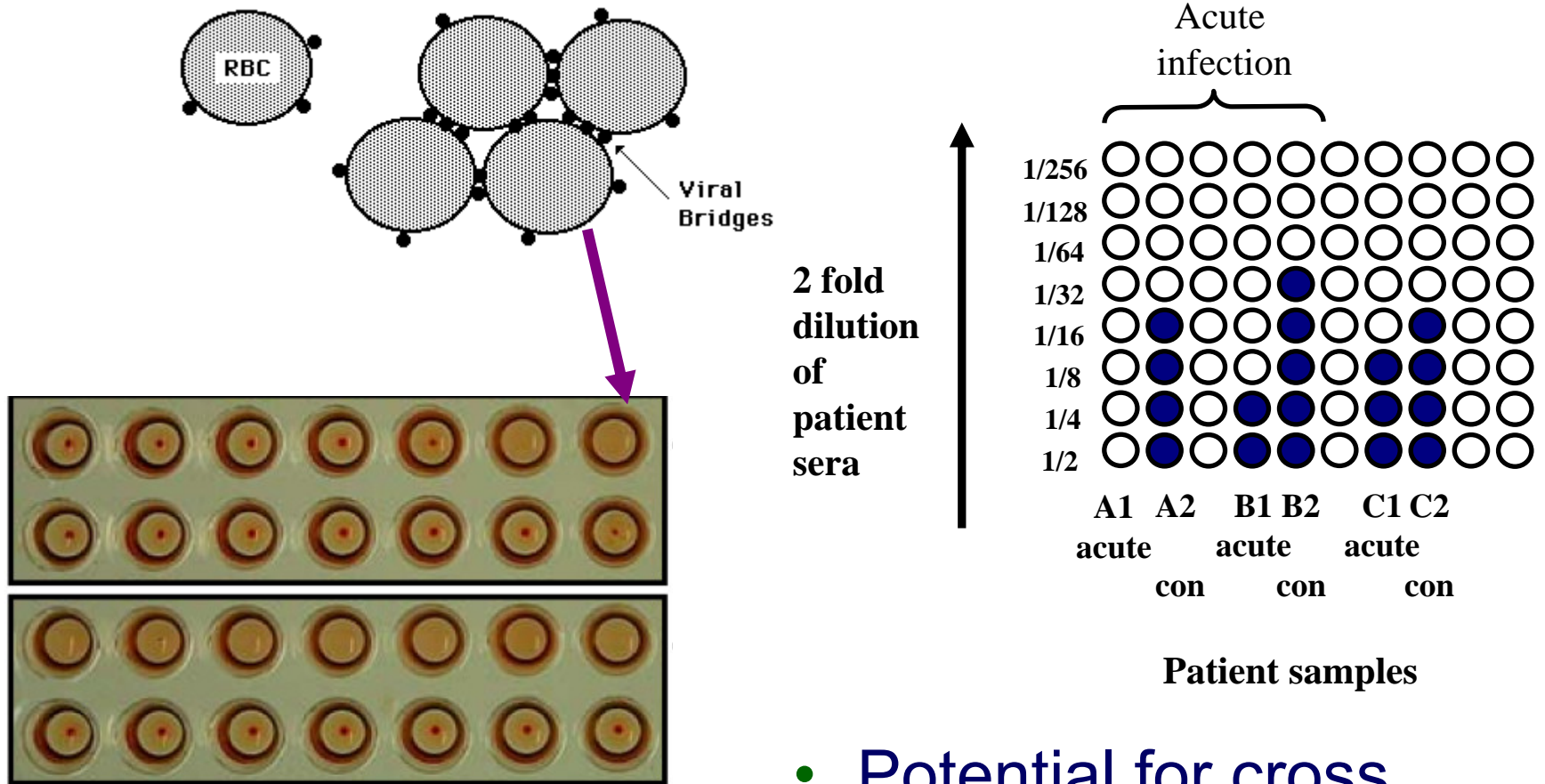


<http://pathmicro.med.sc.edu/mayer/rx-2.jpg>



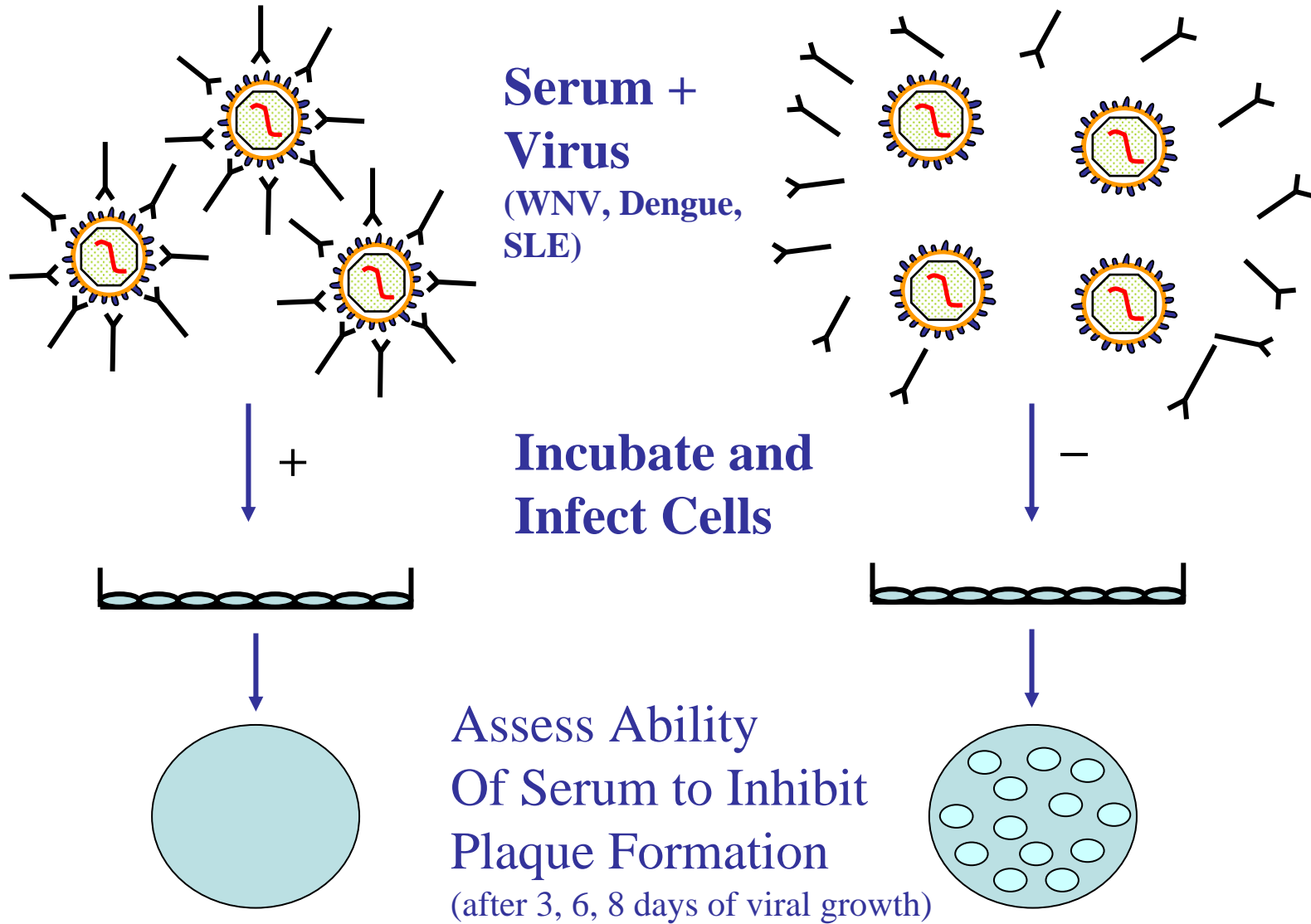
http://www.abbottdiagnostics.co.uk/About_Us/UK/Xchange_19/img/cytomegalovirus_chart.gif

Hemagglutination Inhibition



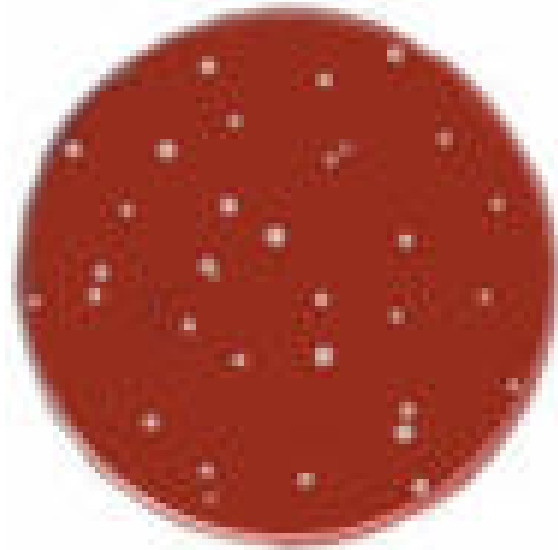
- Potential for cross reactivity

WNV Confirmatory Serology: Neutralization (PRNT) Test



Plaque Reduction Neutralization

- Antibodies in a patient's serum inhibit a virus from infecting a cell monolayer
 - Very specific for virus in question



Caveats to WNV Serologic Testing

- IgM almost always present in Neurological cases
- IgM can be detected 12 months post infection in 36% ; 16 months in 20%
 - Positive test may reflect past infection
- PRNT may be problematic in patients exposed to other flaviviruses

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- Know the typical serologic response
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