Viral Hepatitis

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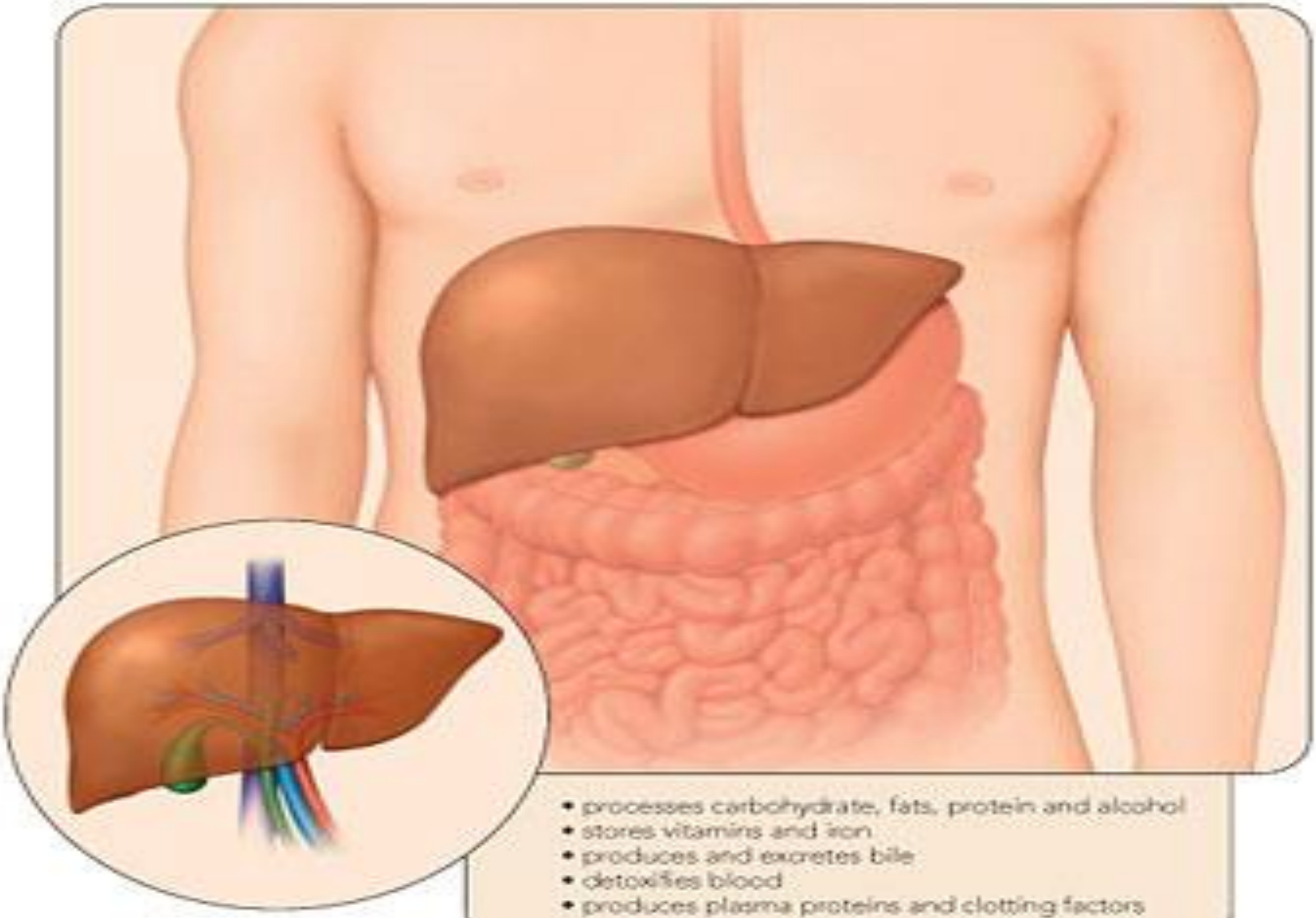
Functions of the Liver

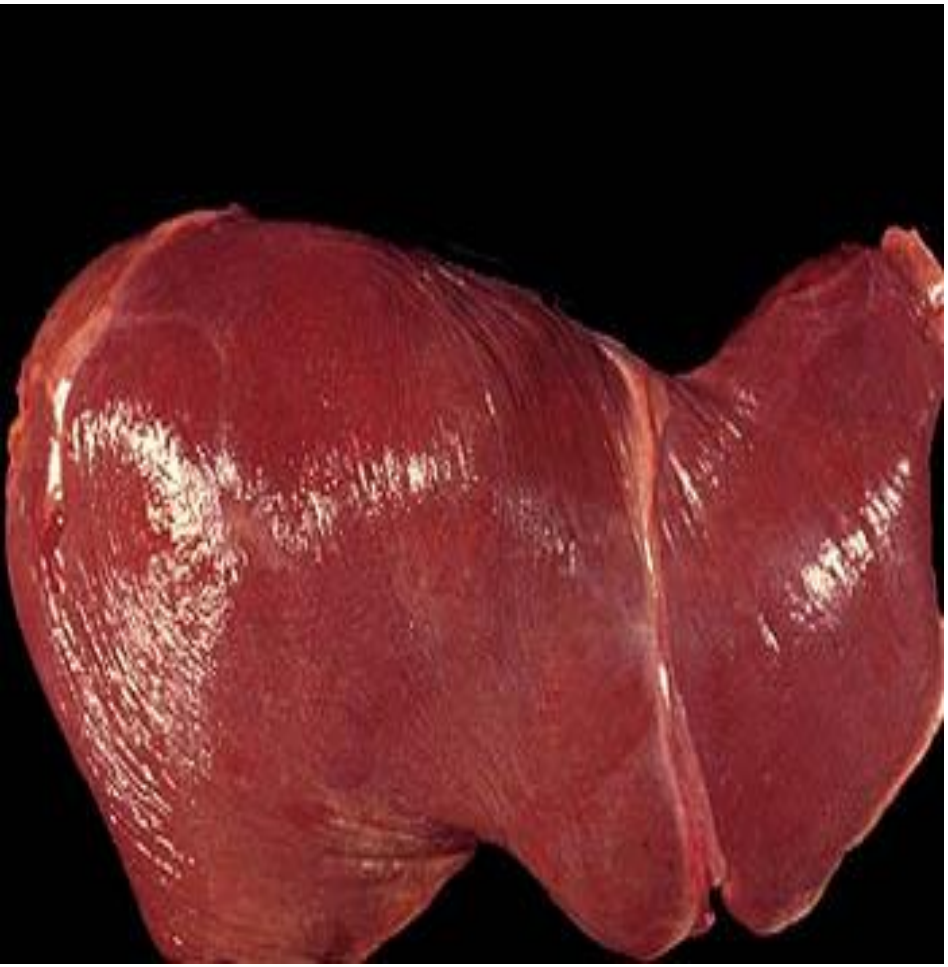


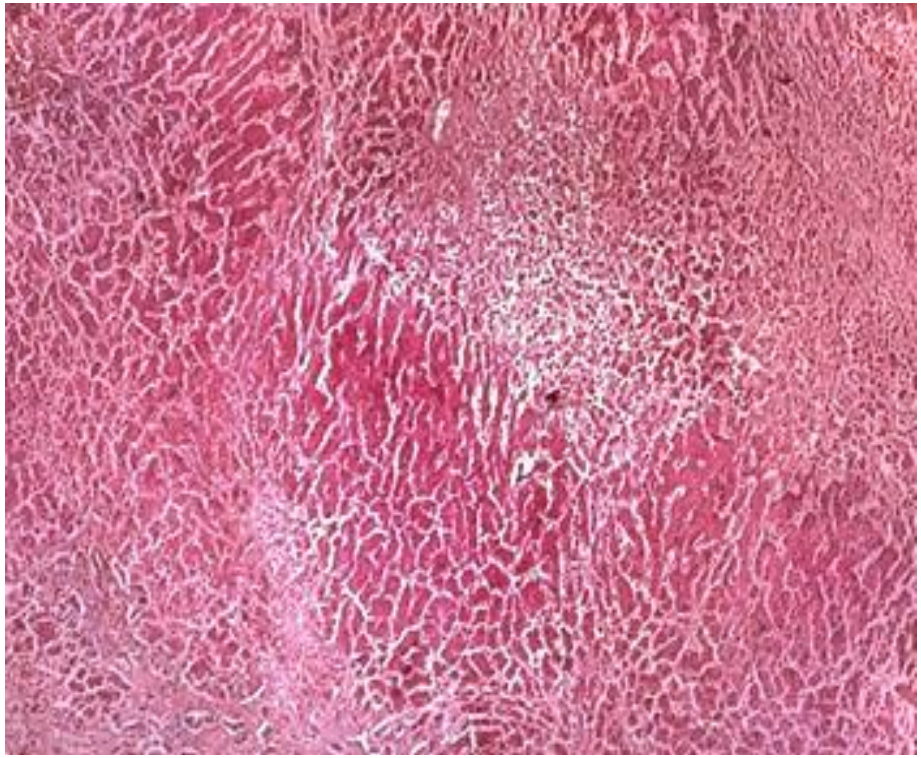
- processes carbohydrate, fats, protein and alcohol
- stores vitamins and iron
- produces and excretes bile
- detoxifies blood
- produces plasma proteins and clotting factors

Hepatitis

- inflammation of the liver
- damage to hepatocytes
- etiology: viruses, bacteria, fungi, parasites, drugs, toxins, hyperthermia, radiation, or excessive alcoholic intake
- fulminant hepatitis

Fulminant Hepatitis





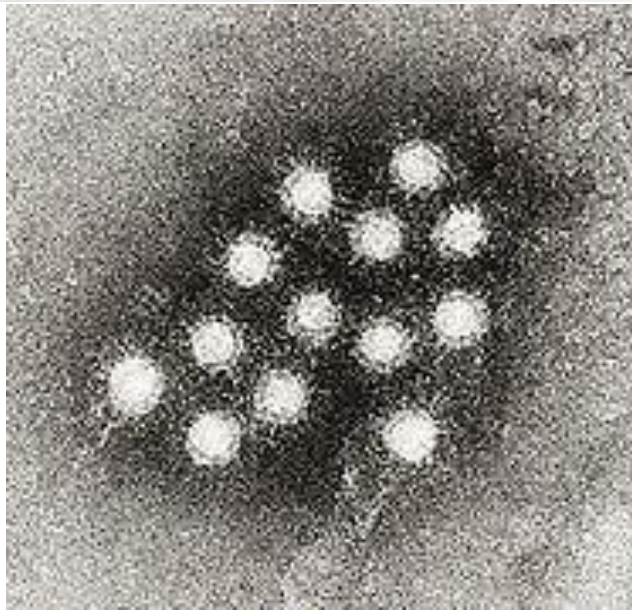
2 Major Clinical Types

- infectious
- serum hepatitis

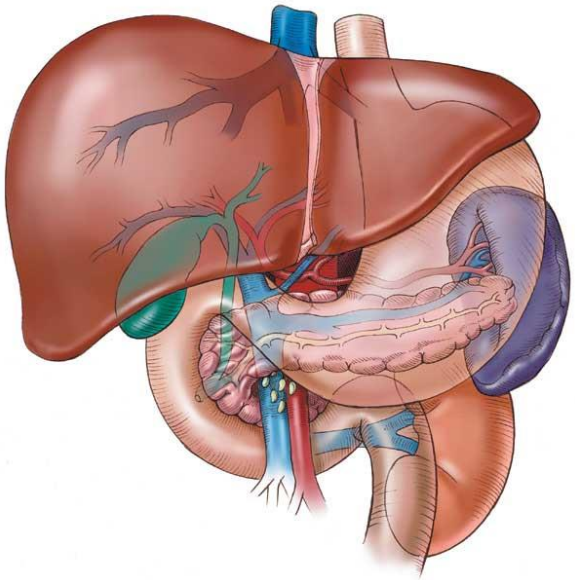
Liver Damage

Hepatitis A

- non enveloped virus; ss RNA;
- Picornaviridae
- fecal oral route
- ether stable, pH. 3.0
- Enterovirus
- IgM → IgG
- Incubation 20-50 days
- recovery: will be 3 weeks to 6 mos
- 2 vaccine (initial shot, booster shot



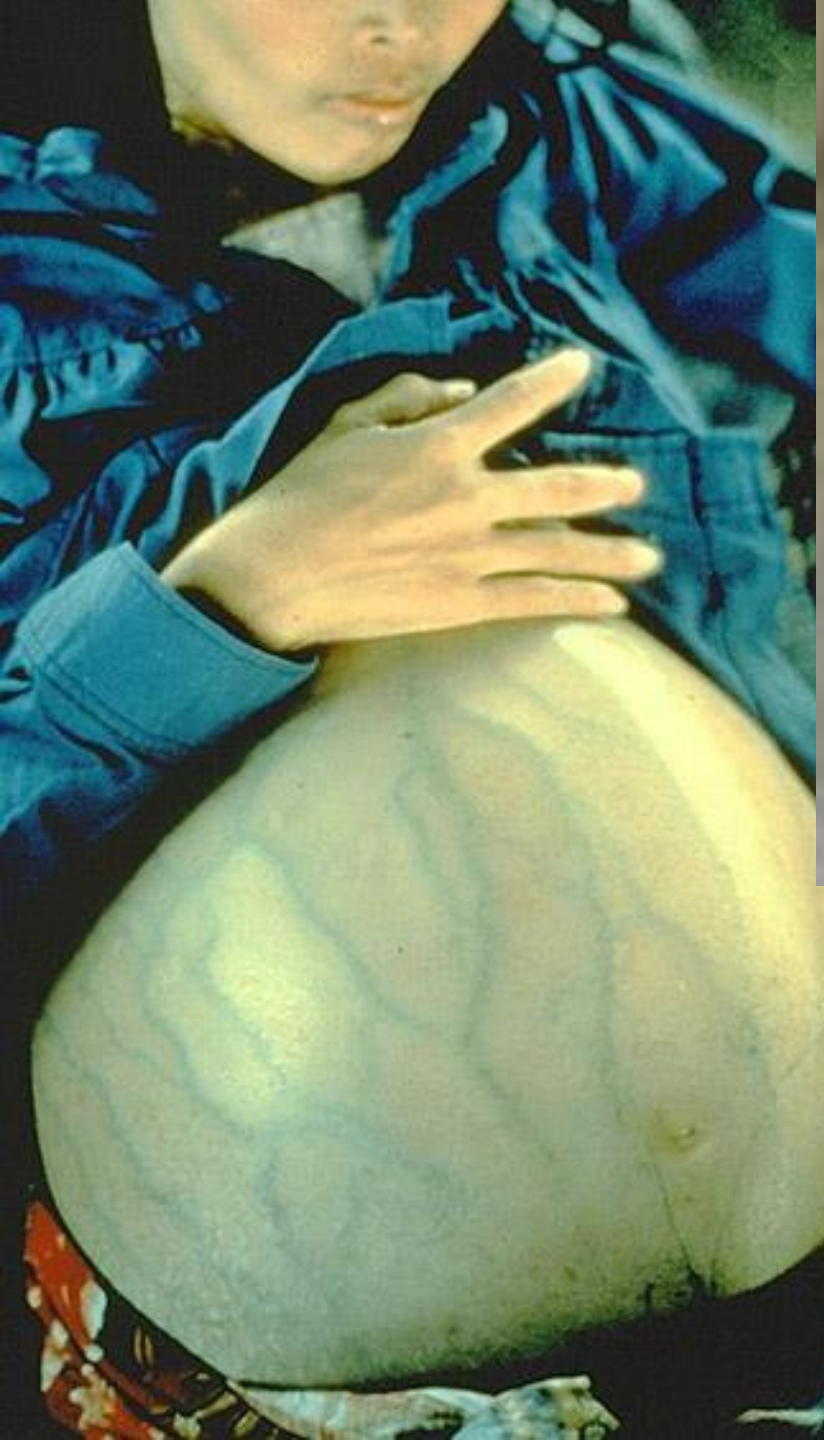
- Following ingestion, HAV enters the bloodstream through the epithelium of the oropharynx or intestine.
- The blood carries the virus to its target, the liver, where it lives and multiplies within hepatocytes and Kupffer cells (i.e., liver macrophages).

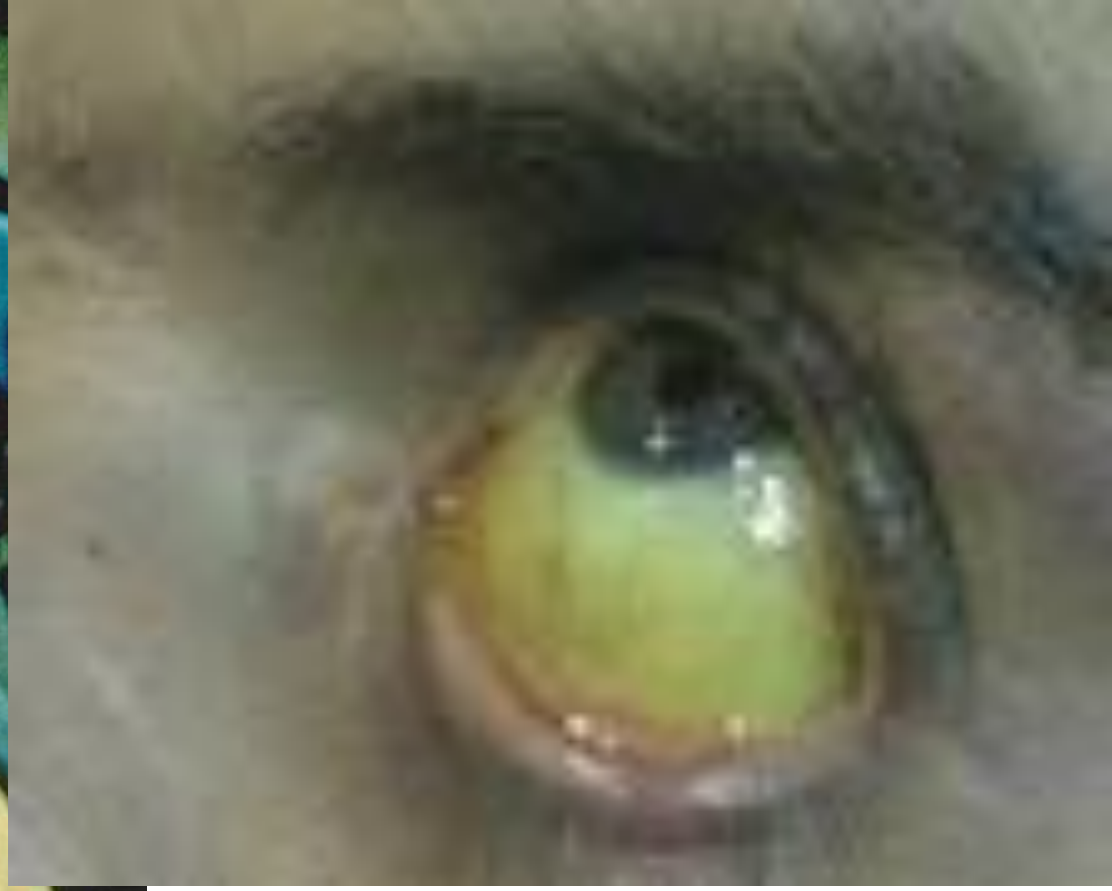


- There is no apparent virus-mediated cytotoxicity, and liver pathology is likely immune-mediated. Virions are secreted into the bile and released in stool.
- HAV is excreted in large quantities approimately 11 days prior to appearance of symptoms or anti-HAV IgM antibodies in the blood. The incubation period is 15-50 days, and mortality is less than 0.5%.

Common Symptoms

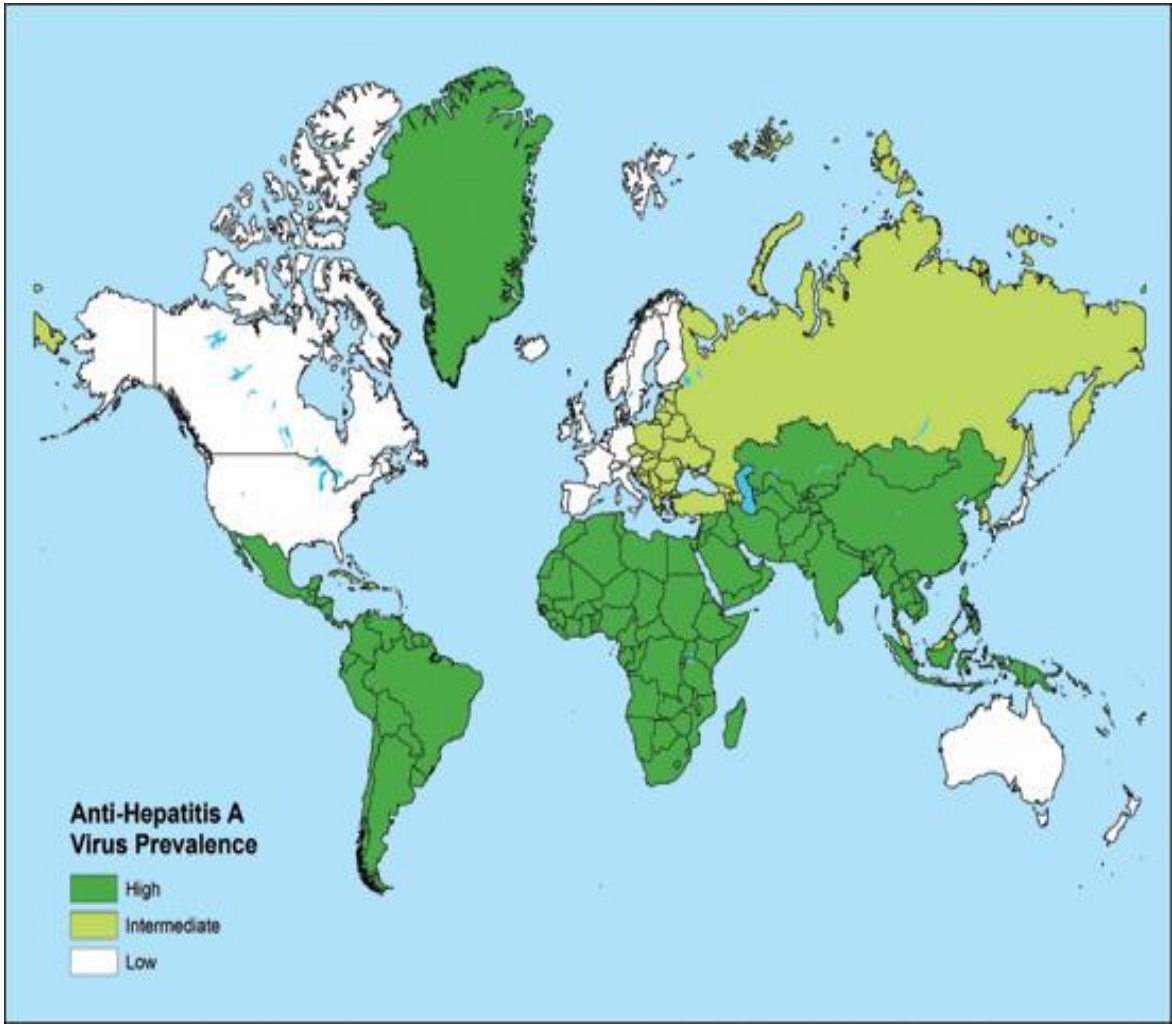
- fatigue
- nausea
- vomiting
- fever; chills
- jaundice
- pain in the liver area
- dark urine
- light colored stool

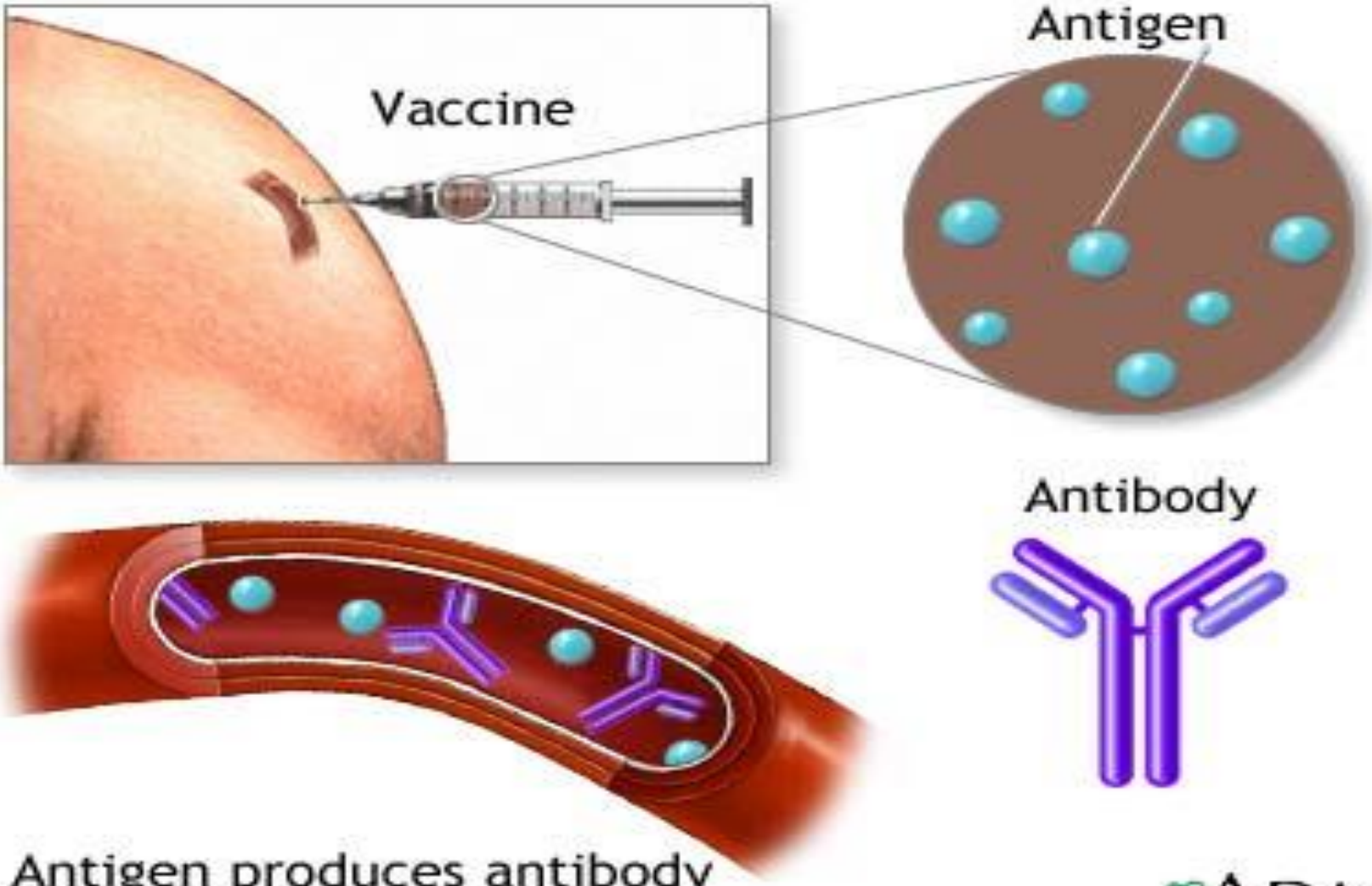




Endemic Area

- countries w/ low standard of living
- Africa
- Asia (except Japan)
- Mediterranean
- Eastern Europe
- Middle East




Vaccine
Antigen
Antibody
Antigen produces antibody
A.D.A.M.

Hepatitis B

- Australian ag
- Hepatotropic virus
- ds DNA → 3 forms

1. spherical disc: 22 nm (blood)
2. filamentous form: 22 nm wide, 50-200 nm long
3. Dane particle: 42 nm diameter, 27 nm nucleocapsid core, surrounded by an outer lipoprotein coat

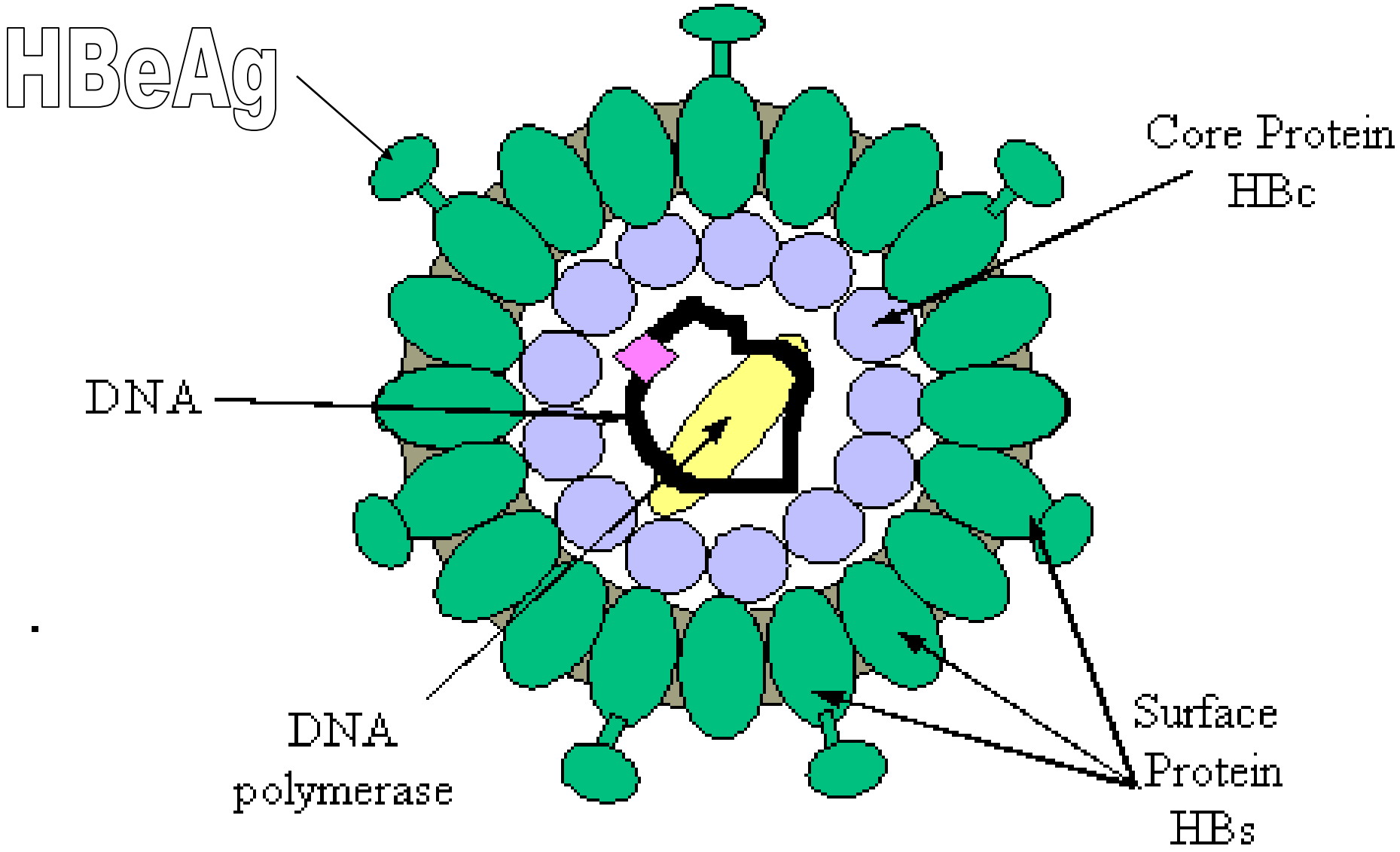
HBV Markers

- HBsAg outer lipoprotein coat: 22nm in diameter, found in body fluids, produced in the cytoplasm of he hepatocyte
- HBcAg: 27 nm in diameter, located in te nuclei
- HBeAg: found in some HBsAg positive sera either bound to Igs or free in solution

  - appears during acute infection

  - marker for infectivity of HBsAg (+) blood

HBV Markers

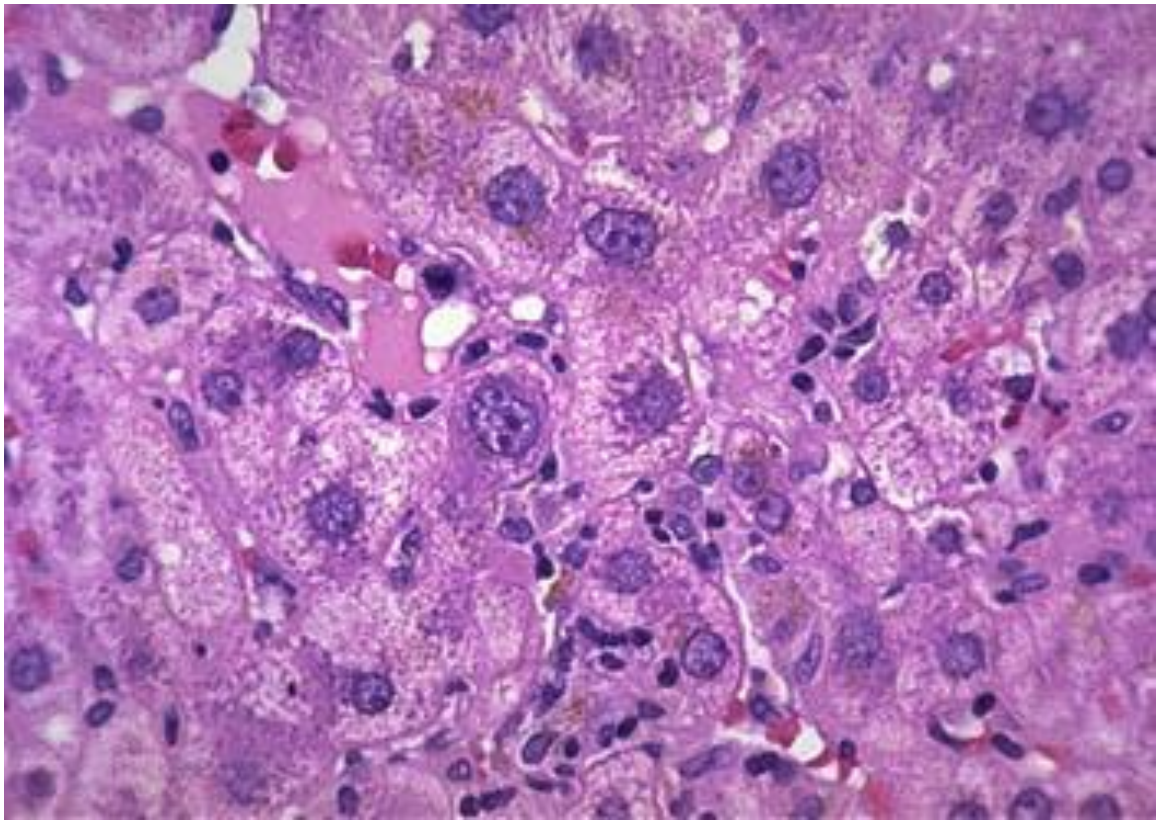

HBeAg
Core Protein
HBc
DNA
DNA
polymerase
Surface
Protein
HBs

Mode of Transmission

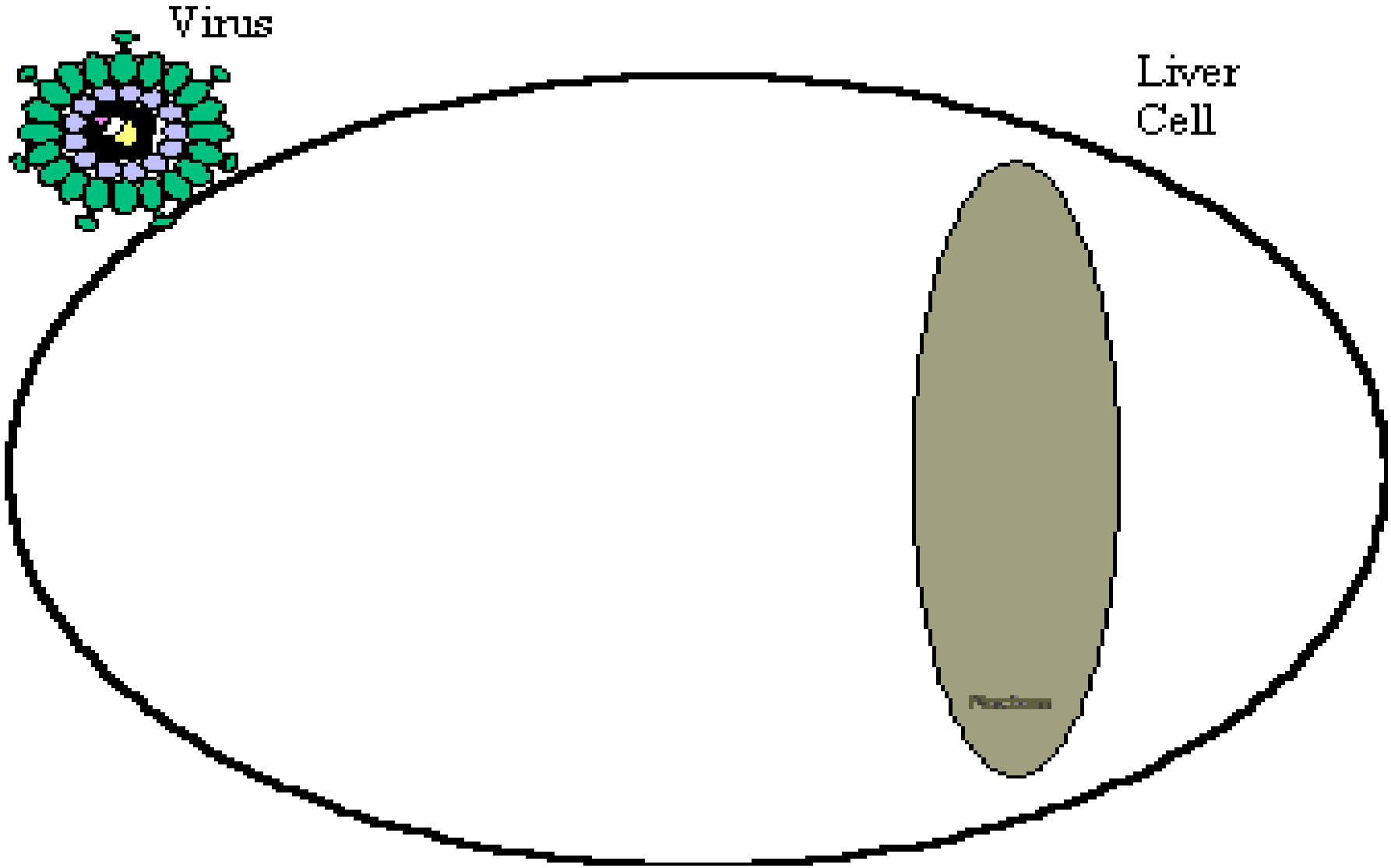
- blood transfusion
- common needles and syringes
- unsterilized dental equipment
- tattoing needles
- sharing of razor and toothbrushes
- sexual contact

Signs and Symptoms

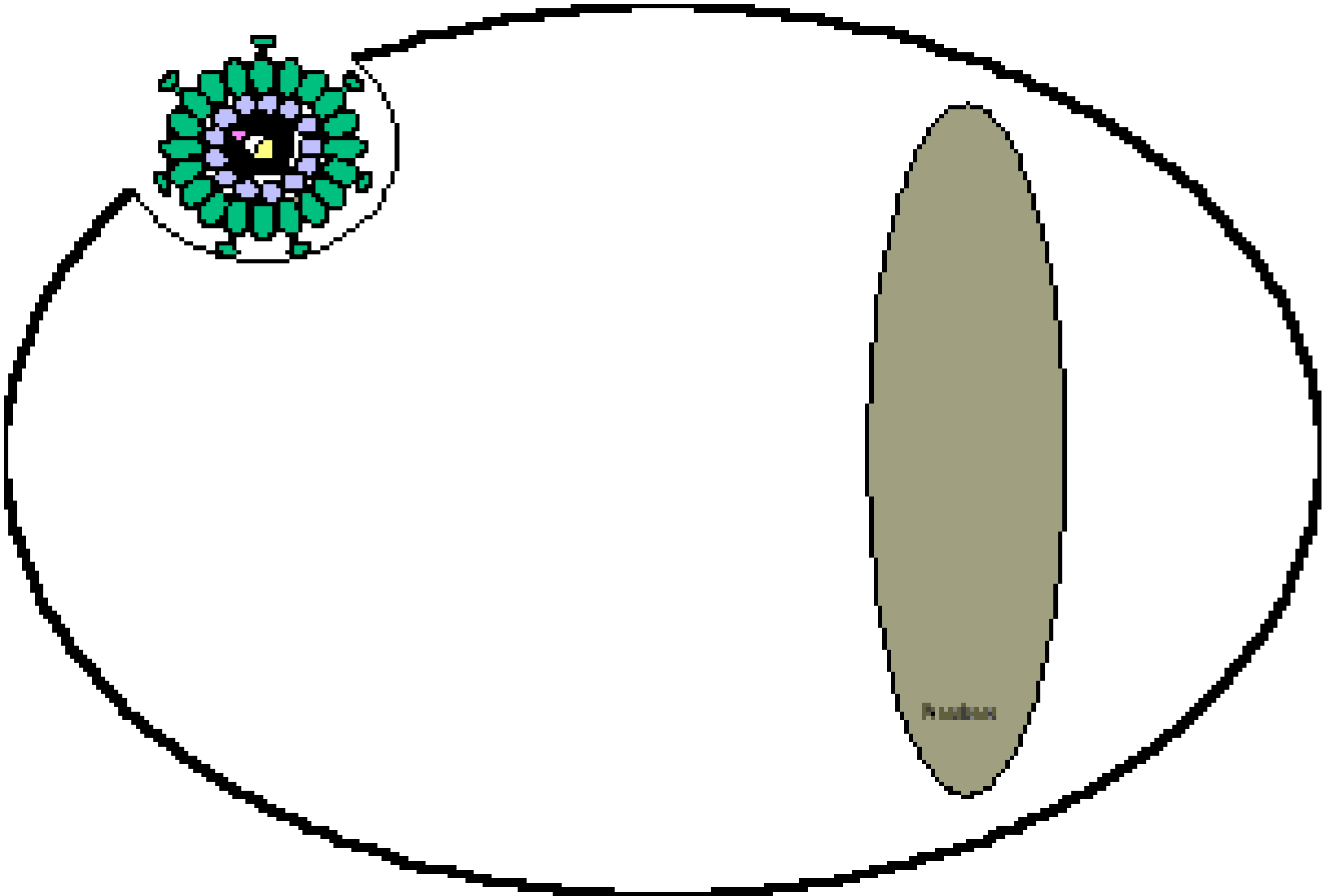
- 10-16 weeks after exposure
- same as Hepa A

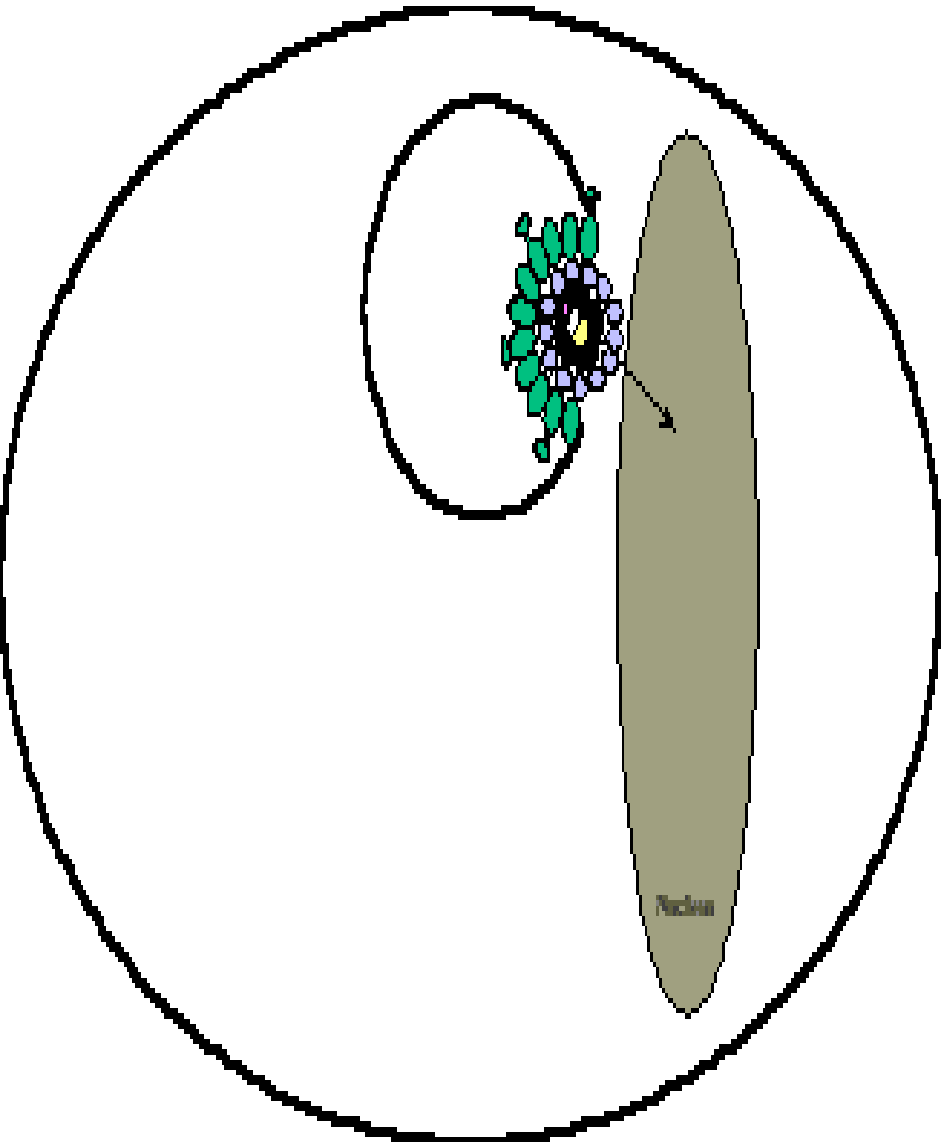


The virus attach to the liver cell membrane



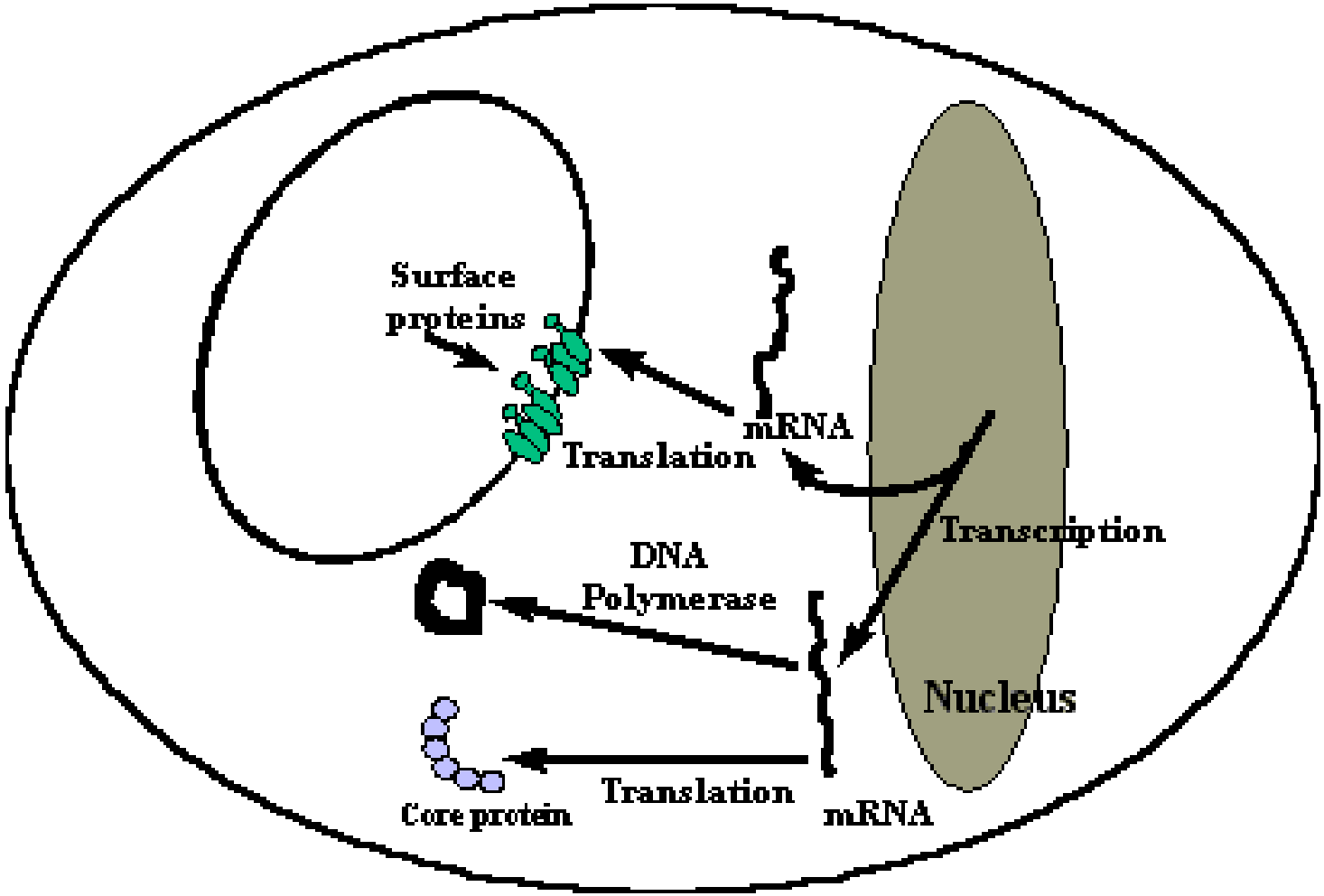
The virus is transported to the liver cell



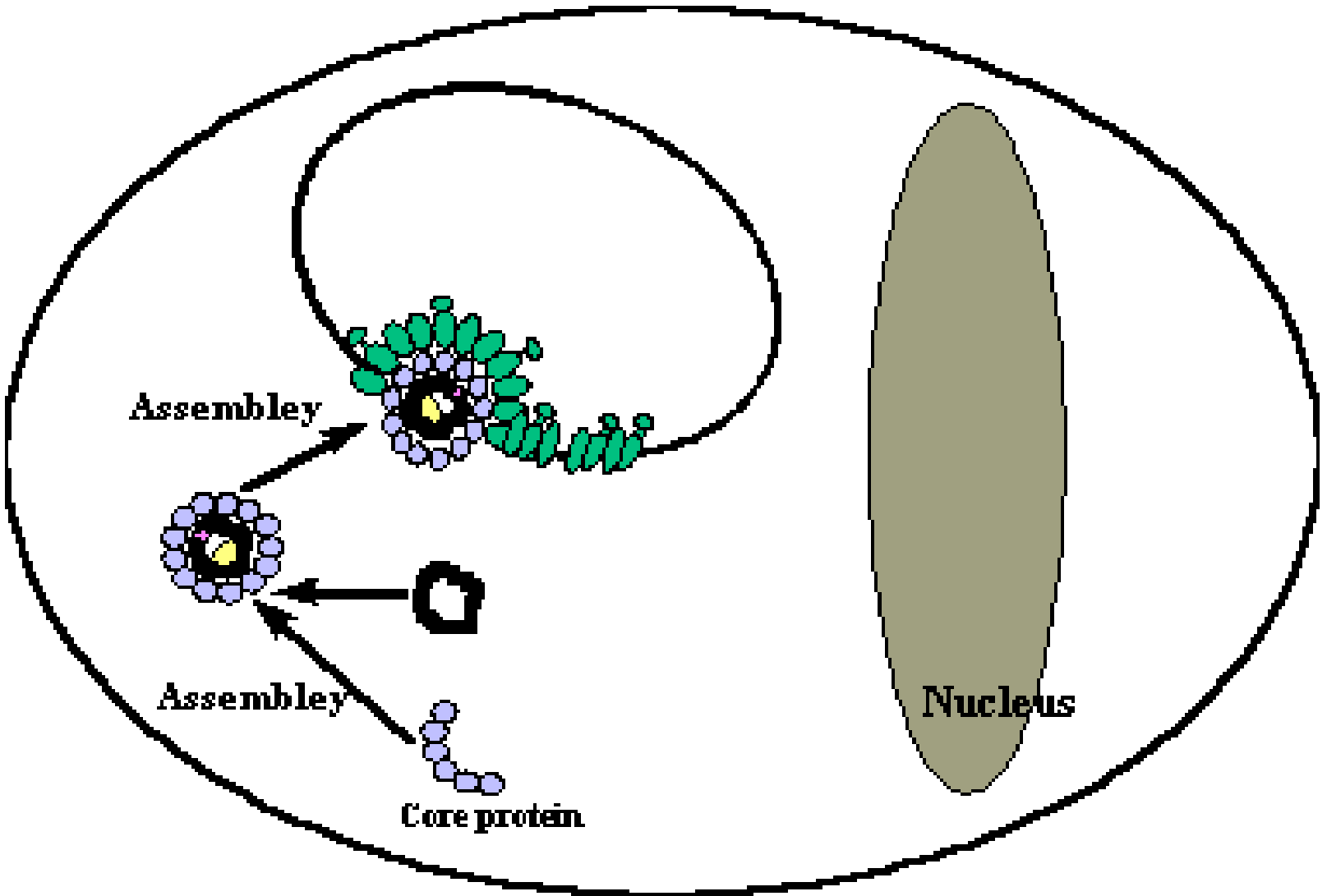


- The core particle then releases it's contents of DNA and DNA polymerase into the liver cell nucleus

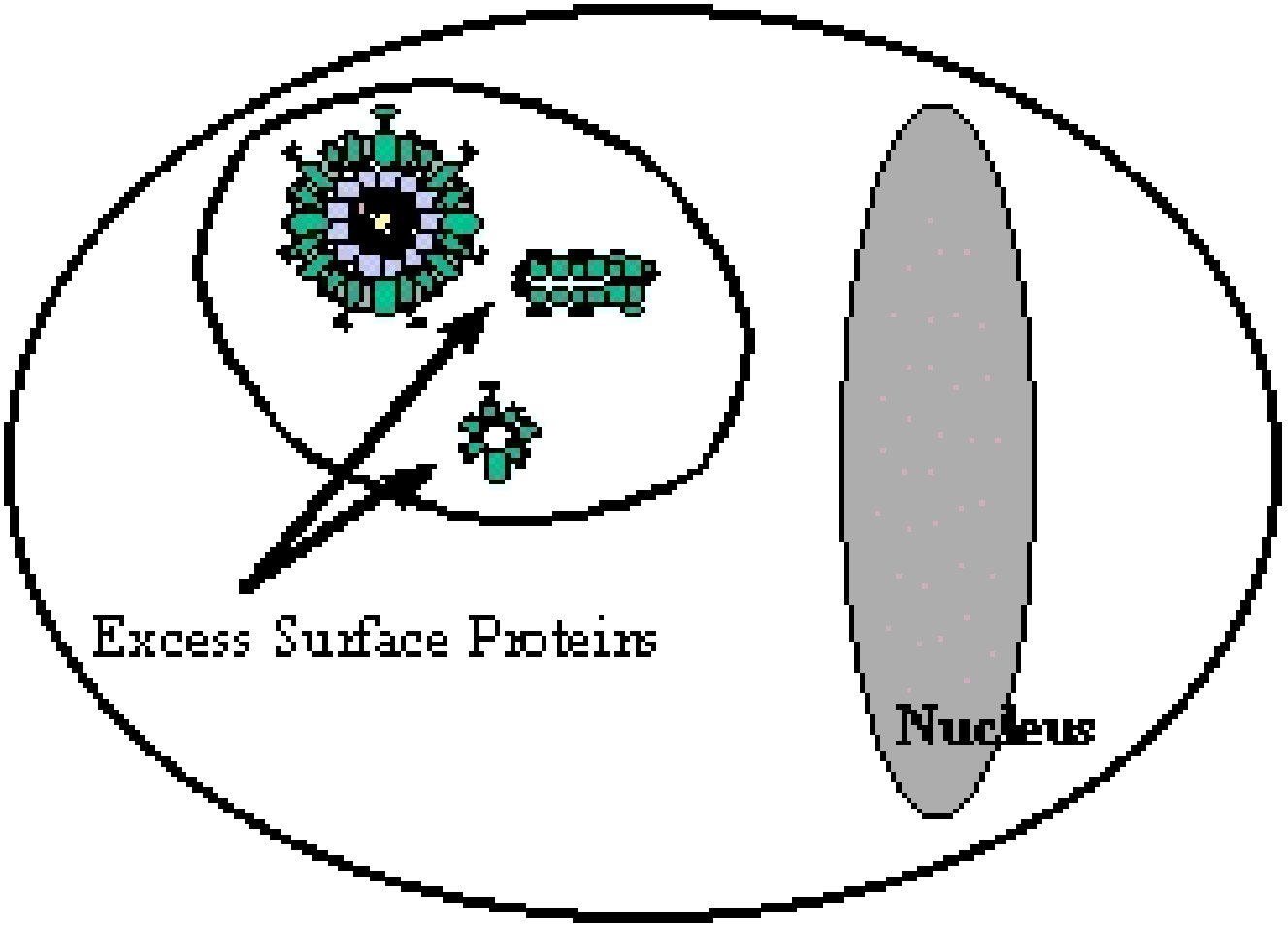
DNA polymerase causes the liver cell to make copies of hepatitis B DNA from messenger RNA.



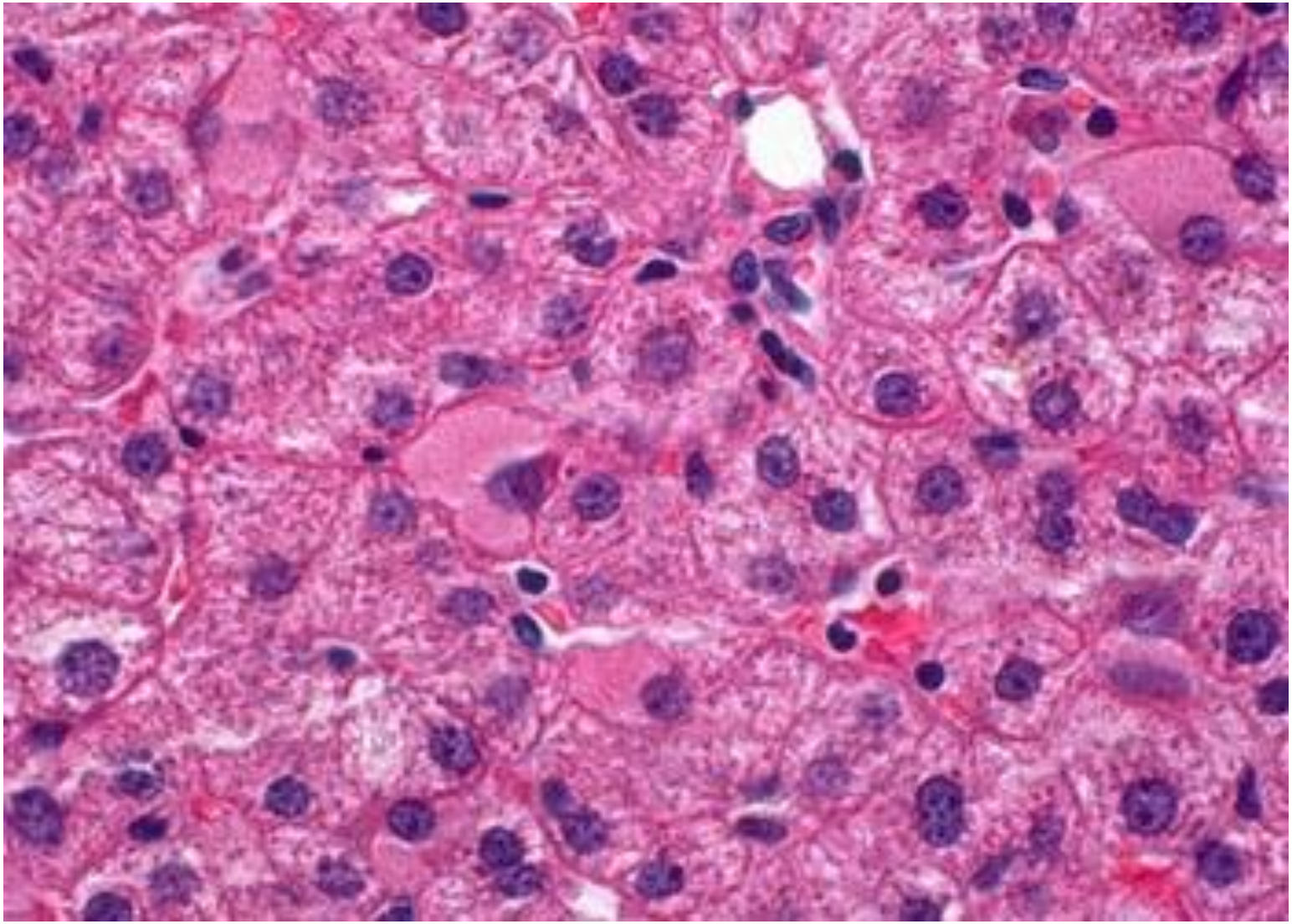
Viral Assembly



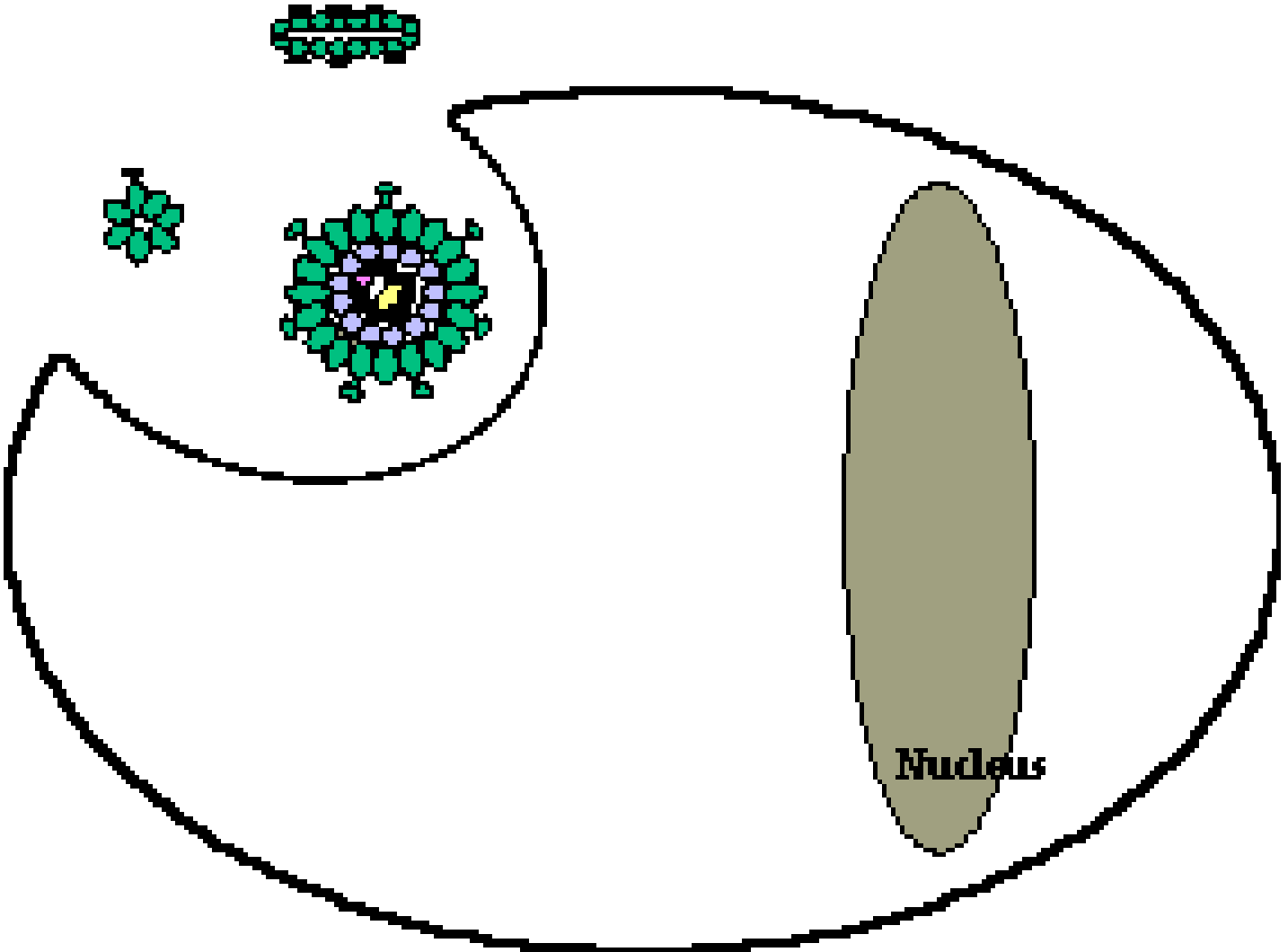
Excess surface protein gives ground glass appearance under the microscope



Ground Glass appearance



Viral release to infect another hepatocyte

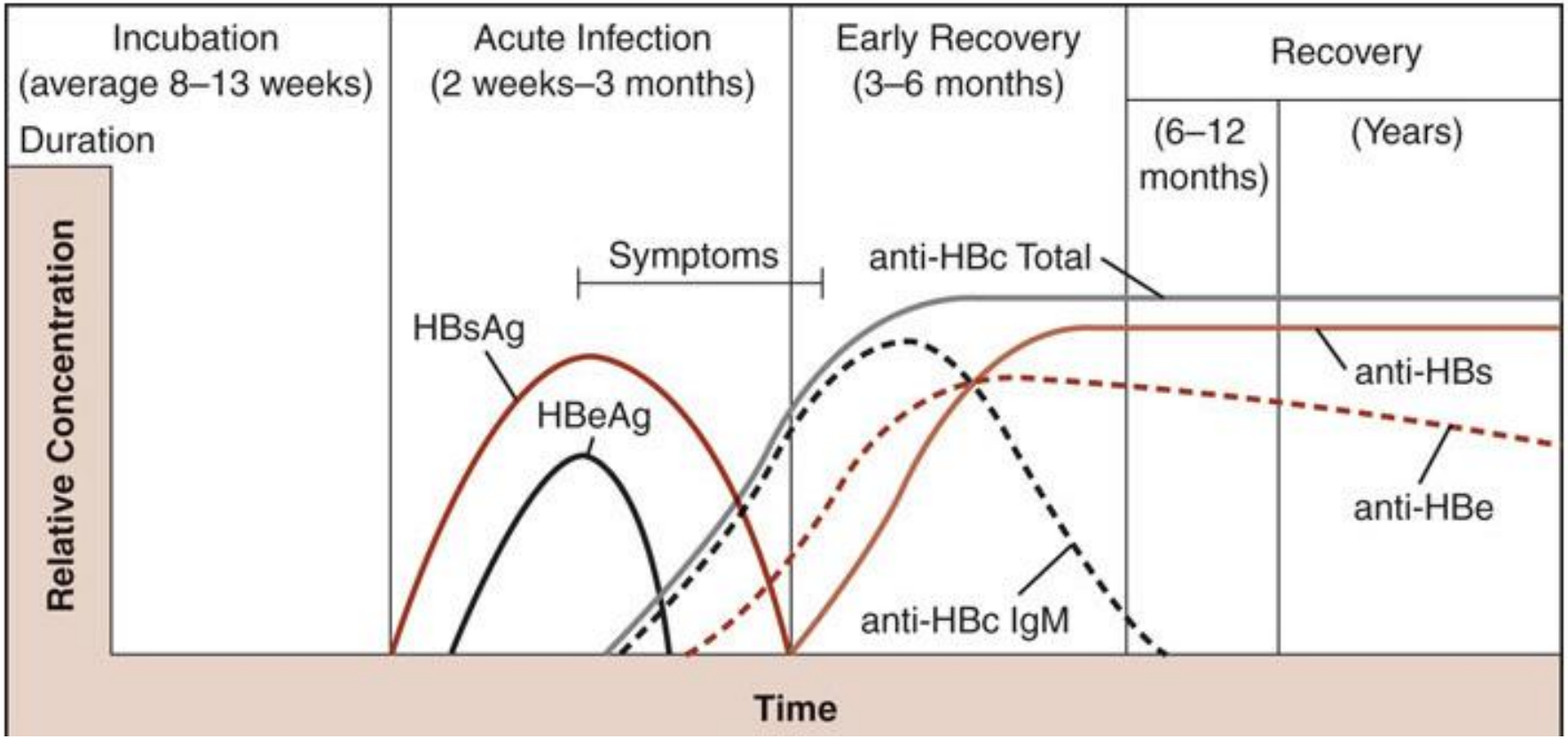


Clinical Pattern

	HBsAg	Anti-HBs	Anti-HBc
Early infection or before acute disease	+	-	-
Late acute infection	+	-	+
Recovery	-	+	+
If low level of nonspecific rxn immunization w/ HBsAg	-	+	-
Window phase; long after infection	-	-	+

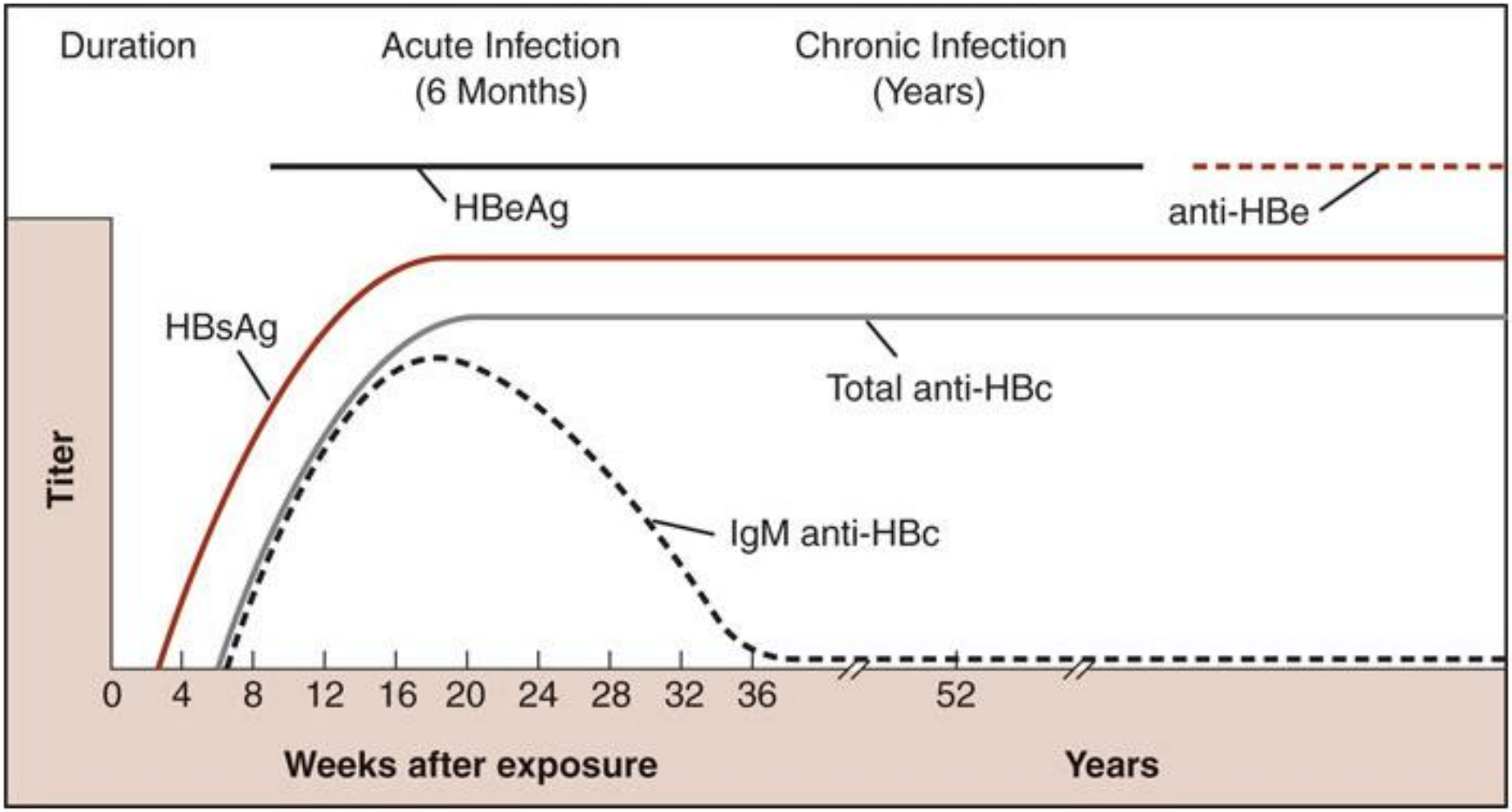
Serology and Molecular Detection of Viral Infections

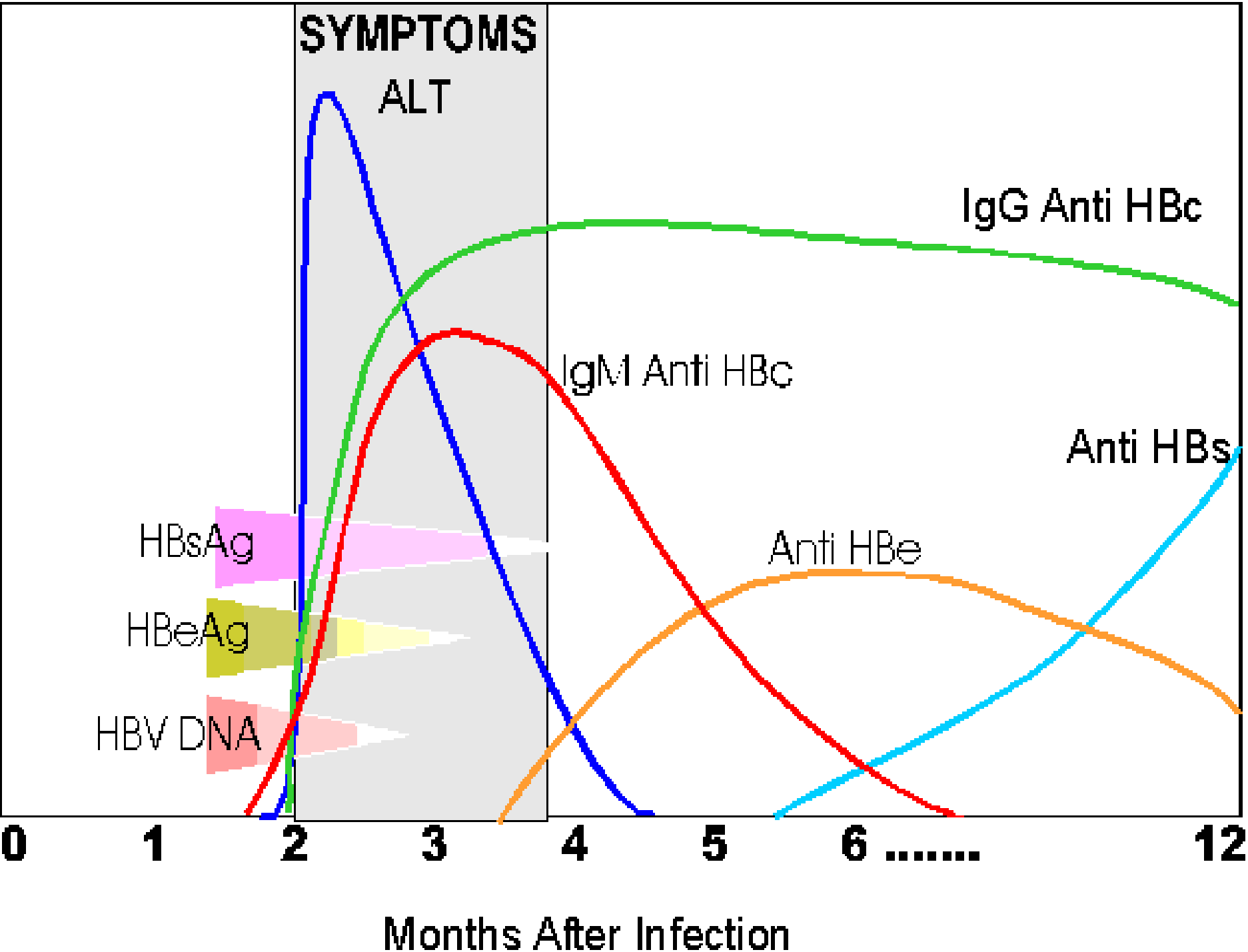
Figure 22-2



Serology and Molecular Detection of Viral Infections

Figure 22-3




SYMPTOMS
ALT
IgG Anti HBc
IgM Anti HBc
Anti HBs
HBsAg
Anti HBe
HBeAg
HBV DNA
0
1
2
3
4
5
6 .....
12
Months After Infection

Serology and Molecular Detection of Viral Infections – Hepatitis B

- The hepatitis Be antigen, or HBeAg, appears shortly after HBsAg and disappears shortly before HBsAg in recovering patients.
- This marker is present during periods of active replication of the virus and indicates a high degree of infectivity.
- The HBcAg is not detectable in serum, because the viral envelope masks it.

Serology and Molecular Detection of Viral Infections – Hepatitis B

- The first antibody to appear is IgM antibody to the core antigen, or IgM anti-HBc.
- This antibody indicates current or recent acute infection.
- The appearance of antibodies to the HBe antigen, or anti-HBe, occurs shortly after the disappearance of HBeAg and indicates that the patient is recovering from HBV infection.

Serology and Molecular Detection of Viral Infections – Hepatitis B

- Antibodies to HBsAg, or anti-HBs, also appear during the recovery period of acute hepatitis B, a few weeks after HBsAg disappears.
- These antibodies persist for years and provide protective immunity. Anti-HBs are also produced after immunization with the hepatitis B vaccine.

Serology and Molecular Detection of Viral Infections – Hepatitis B

- Serological markers for hepatitis B are most commonly detected by enzyme immunoassay and chemiluminescent immunoassay.
- An example of an immunoassay for detecting HBsAg is shown in **Figure 22-4**.

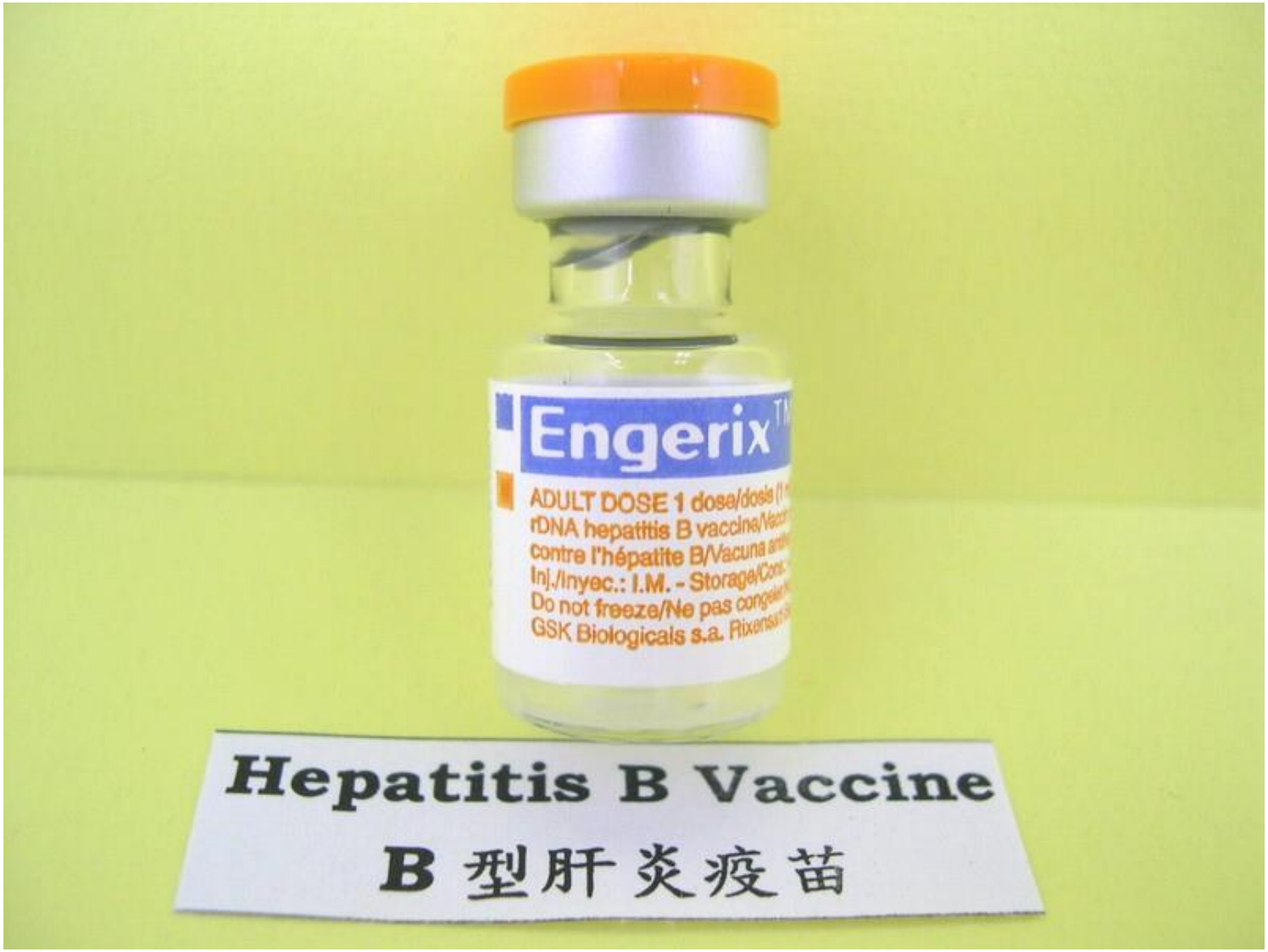
When do we need vaccine?

Marker	**Test**	**Interpretation**	**Vaccine?**
HBsAG Anti-HBc	+ +	Patient is a carrier	No
HBsAG Anti-HBc	- +	Exposure; developed immunity	No
HBsAG Anti-HBc	- -	Susceptible to Hepa B	Yes
HBsAG Anti-HBs	+ -	Patient is an infected carrier	No
HBsAG Anti-HBs	- +	Developed natural immunity; successful vaccination	No
HBsAG Anti-HBs	- -	Patient is susceptible	Yes

3 Doses Vaccination

- 1st Dose
  - infants born to infected mother
  - within 12 hrs
  - infant 1-2 months
- 2nd Dose
  - 1 month later
- 3rd Dose
- - 6 months after 1st dose

Engerix Vaccine



Recombivax


100mg/mL
NDC 0007-4407-01
ARGATROBAN
INJECTION
Rx only
For I.V. Infusion Only
Dilute Prior to Use
2.5 mL Single Dose Vial
SB SmithKline Beecham

Treatment

- alpha interferon
- nucleoside analogue
- lamivudine

Prevention

- HBV vaccine
- screening pregnant women
- avoid unprotected sexual contact
- avoid sharing of needles, razor etc

Serology and Molecular Detection of Viral Infections

- **Hepatitis C virus (HCV)** is transmitted mainly by exposure to contaminated blood, with IV drug use being the main source of infection.
- It is the cause of the majority of infections previously classified as “nonA-nonB” before the discovery of HCV in 1989.

Serology and Molecular Detection of Viral Infections – Hepatitis C

- Blood transfusion was also a major source of infection before 1992, when routine screening of blood donors for HCV antibody was implemented.
- Other risk factors for acquiring hepatitis C include organ transplantation before 1992, occupational exposures to contaminated blood, chronic hemodialysis, intranasal cocaine use, body piercing, and tattooing.

Serology and Molecular Detection of Viral Infections – Hepatitis C

- Sexual transmission of HCV is thought to be less common but is higher in those who have had multiple sex partners or a history of sexually transmitted diseases.
- Perinatal transmission has been estimated to occur at a rate of about 6 percent.

Serology and Molecular Detection of Viral Infections – Hepatitis C

- Although the majority of infections are asymptomatic, the infection is problematic, because about 85 percent of persons develop **chronic infection**, which leads to cirrhosis in about 20 percent of these individuals.
- HCV is an enveloped, single-stranded, positive-sense RNA virus belonging to the family Flaviviridae and the genus *Hepacivirus*.

Serology and Molecular Detection of Viral Infections – Hepatitis C

- Serological tests involve detection of HCV IgG antibody by third-generation enzyme immunoassays or chemiluminescent immunoassay methods, which use recombinant and synthetic antigens.
- Improvements in the serological assays for anti-HCV have enabled antibodies to be detected earlier than previous methods—about 4 to 6 weeks after infection.

Serology and Molecular Detection of Viral Infections – Hepatitis C

- While the specificity of these methods is excellent, false-positive results may occur due to cross-reactivity present in persons with other viral infections or autoimmune disorders.
- Any positive results from an anti-HCV screening test should be confirmed.
- The traditional confirmatory method was the recombinant immunoblot assay (RIBA), which detects antibodies to different HCV antigens.

Serology and Molecular Detection of Viral Infections – Hepatitis C

- However, RIBA has been replaced in many laboratories by molecular methods, which are more sensitive and less labor intensive.
- Quantitative tests are performed by RT-PCR, real-time PCR, or branched DNA amplification (bDNA).

Serology and Molecular Detection of Viral Infections – Hepatitis C

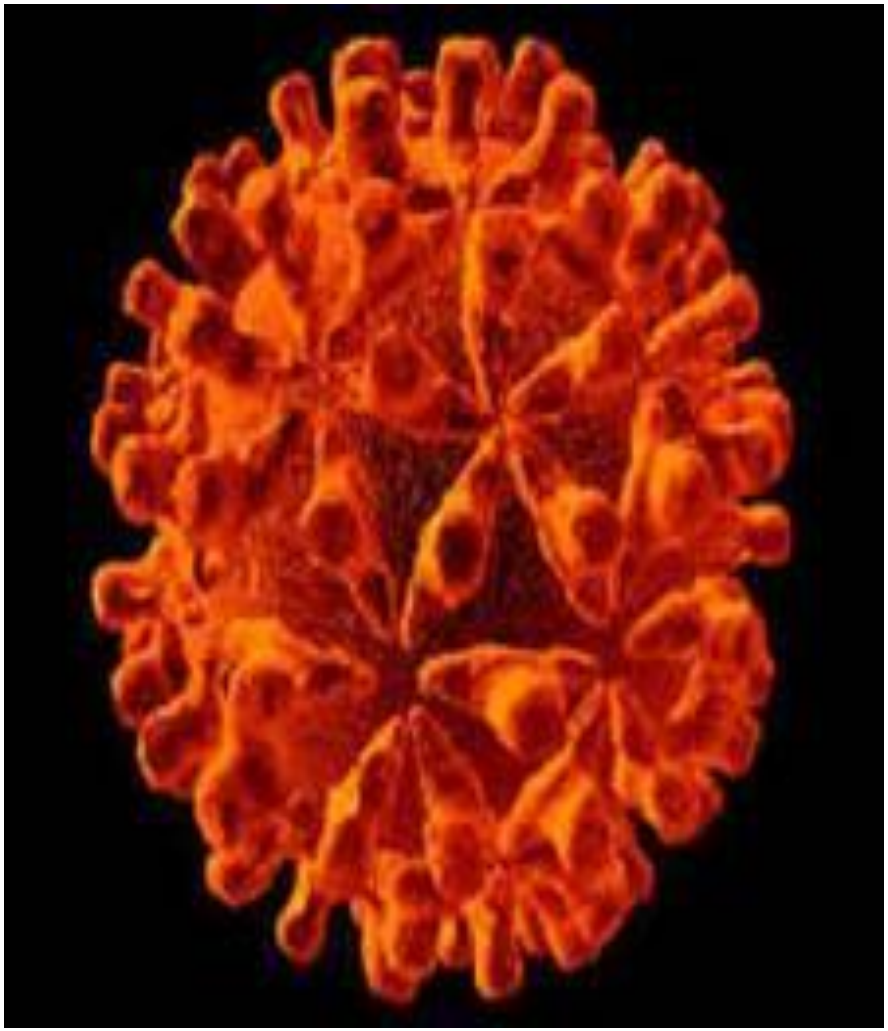
- They are used to monitor the amount of HCV RNA, or “viral load,” carried by patients before, during, and after antiviral therapy.
- Another type of molecular assay for HCV is the genotyping test, which determines the exact genotype and subtype of the virus responsible for the patient’s infection.

Serology and Molecular Detection of Viral Infections – Hepatitis C

- Genotyping tests are ideally performed by sequence analysis, although other methods, including a subtype-specific RT-PCR and a line-probe assay, have also been developed.
- The patient's HCV genotype helps determine optimal treatment in terms of the dose of antiviral drugs administered and the duration of therapy.

Non A Non B (Hepatitis C)

- post transfusion hepatitis
- ALT elevation among blood donors
- minimal clinical manifestations
- liver biopsy -> chronic liver disease
- 6-10 weeks incubation
- MOT: same as Hepa B

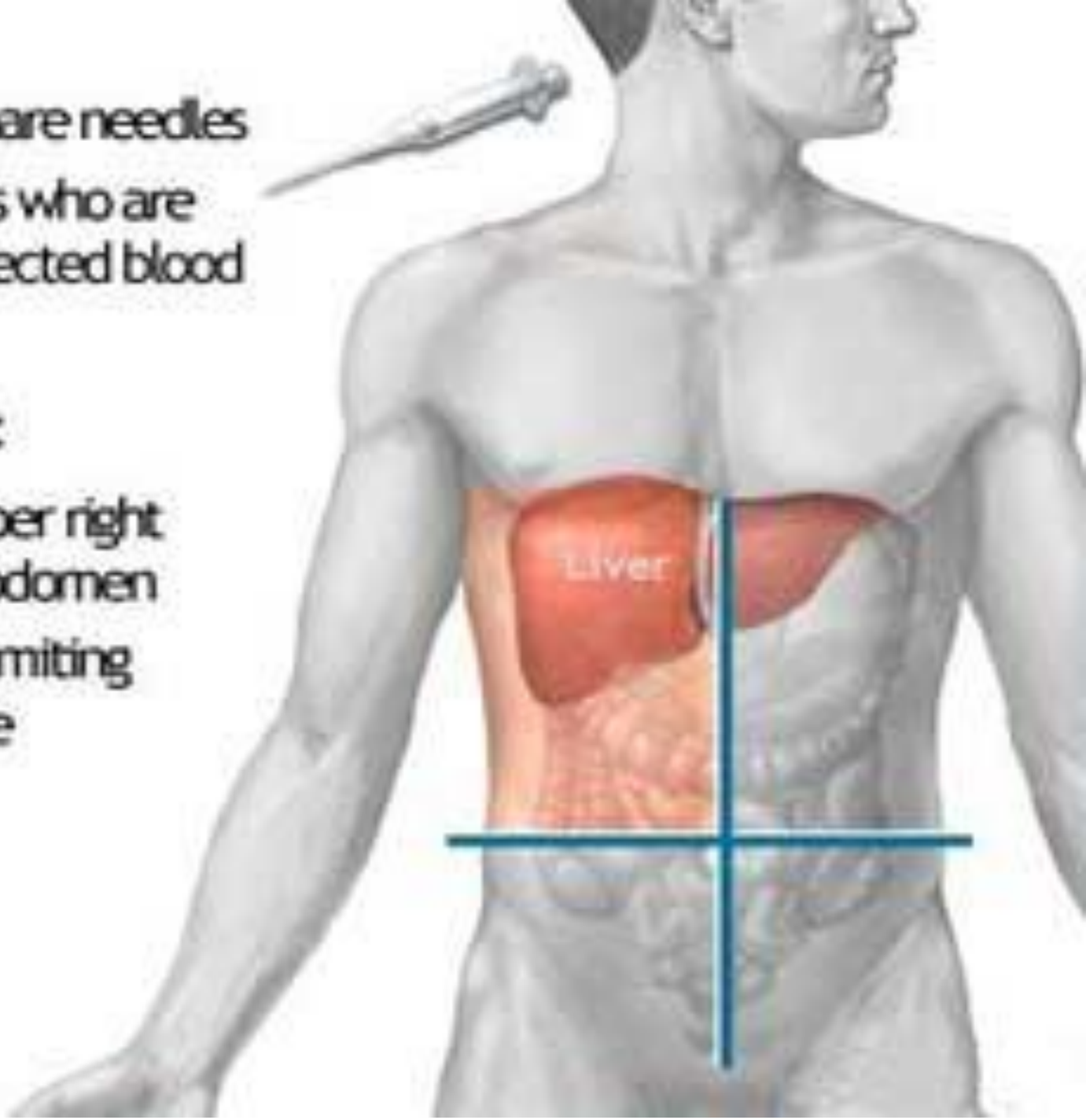


Risk factors:

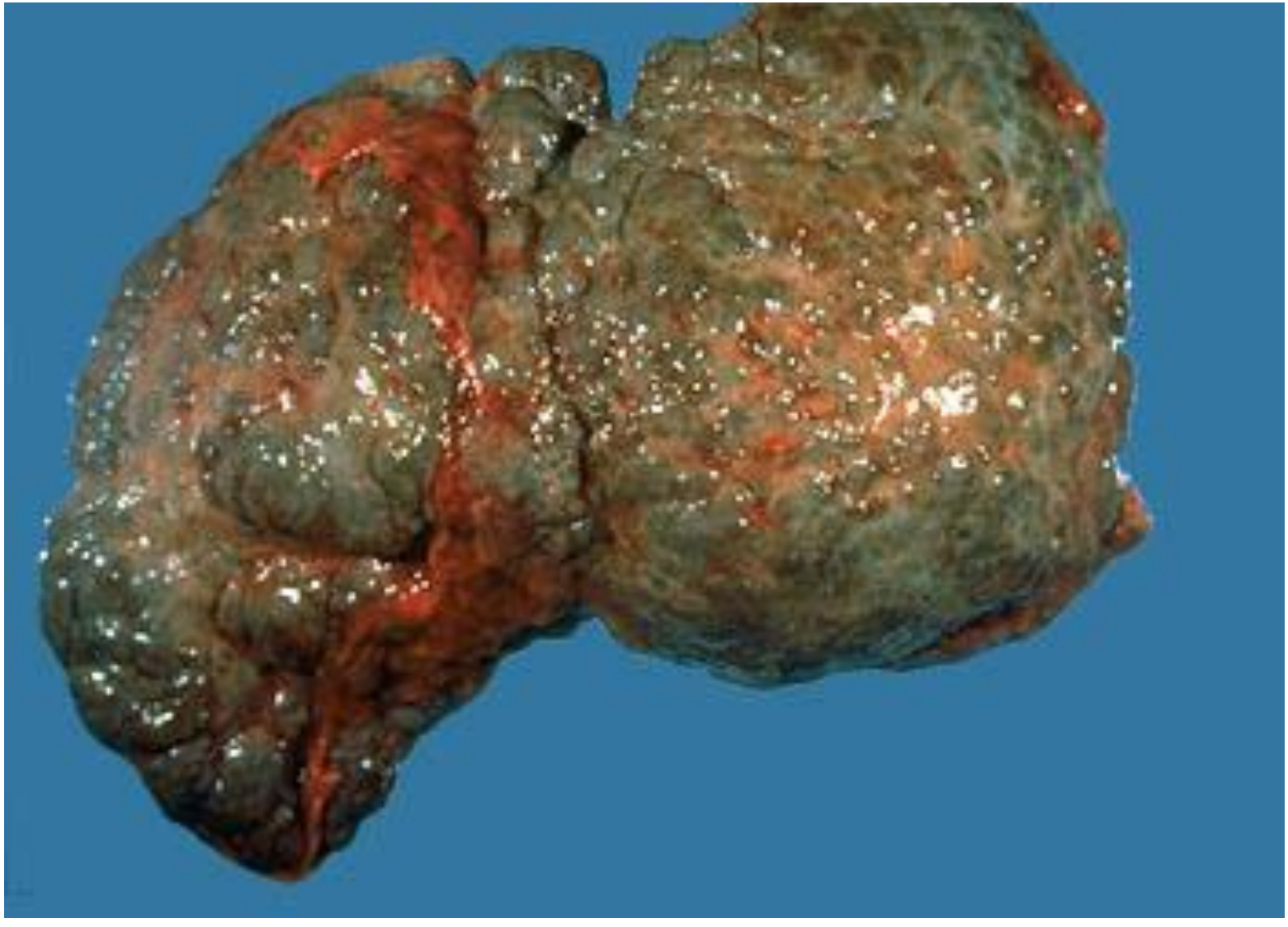
- people who share needles
- health workers who are exposed to infected blood

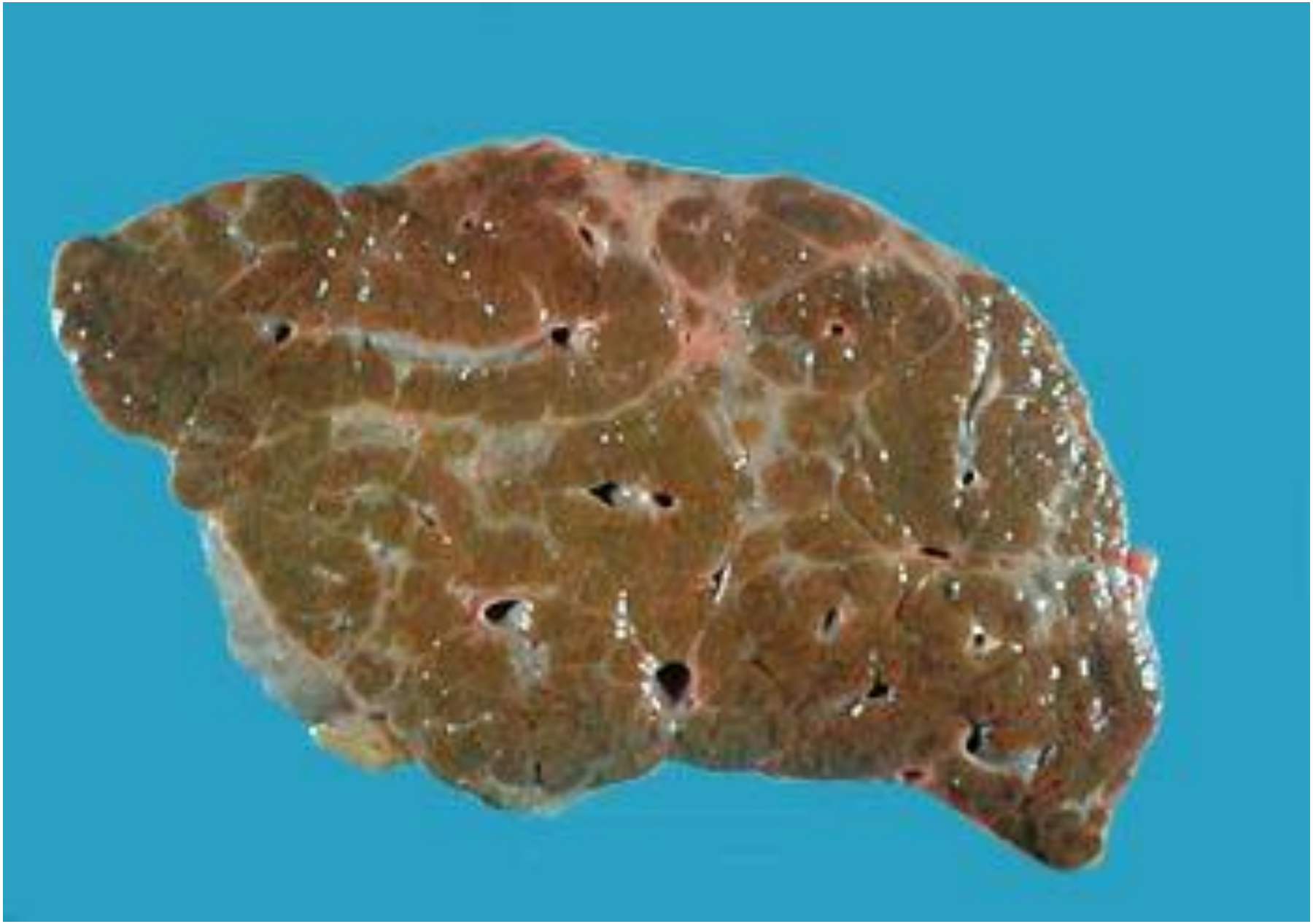
Possible symptoms:

- pain in the upper right quadrant of abdomen
- nausea and vomiting
- loss of appetite
- jaundice
- fatigue
- itching

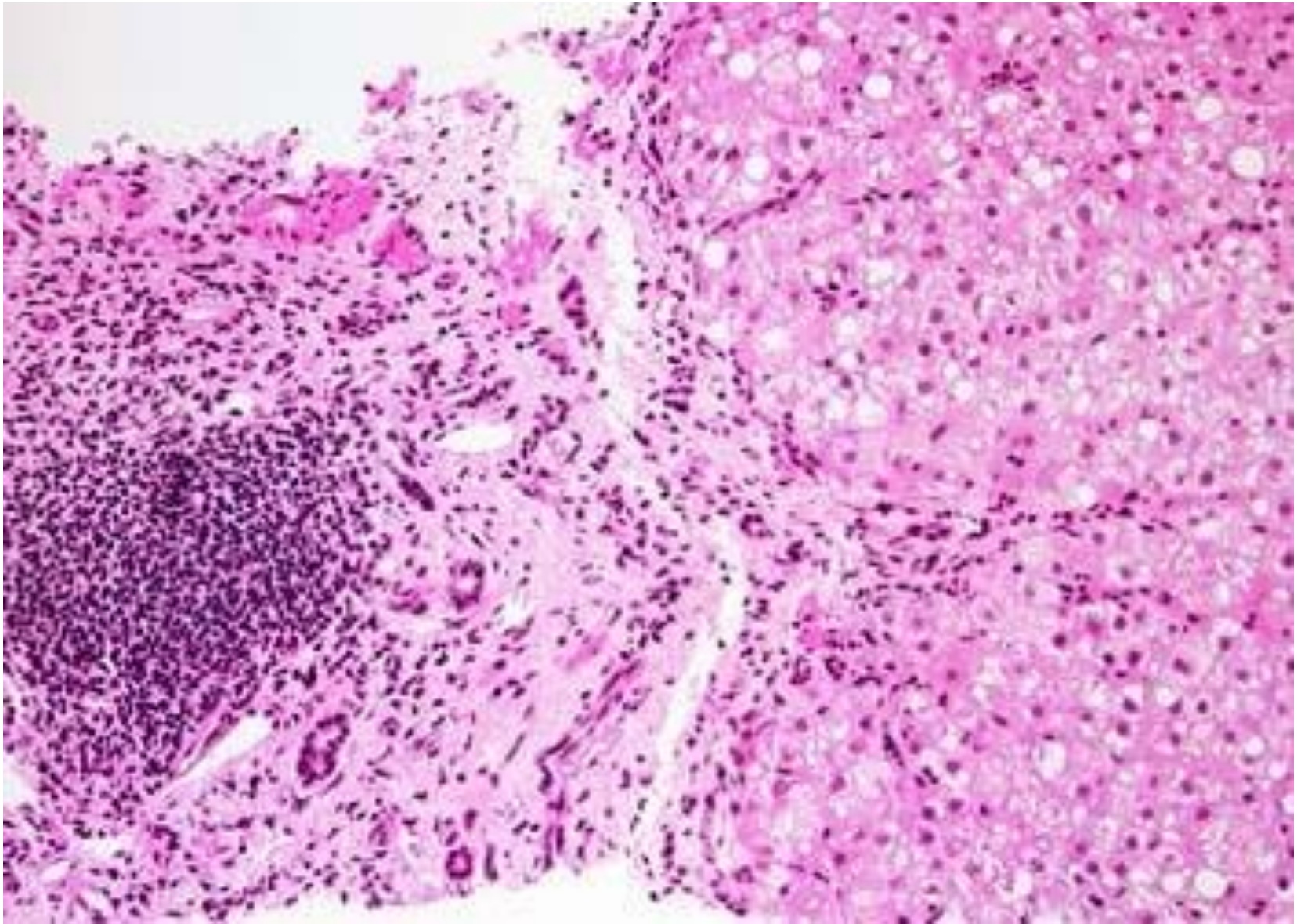


Liver cirrhosis: chronic viral hepatitis





Chronic Viral Hepatitis C



Cure

- Single Agent: Sovaldi (sofosbuvir)
- Combination: Harvoni (ledipasvir and sofosbuvir)
- Gilead
- Cost (2015): USD 80,000.00 = PhP 3.6M

Hepatitis D

- Delta Hepatitis
- ss RNA; spherical in shape 36 nm
- requires helper function with HBV helper virus
- can occur:

  1. co-infection with acute HBV infection
  2. super infection with chronic HBV infection

Serology and Molecular Detection of Viral Infections

- **Hepatitis D, also known as delta hepatitis,** is a parenterally transmitted infection that can occur only in the presence of hepatitis B.
- This is because HDV is a defective virus that requires HBV for its replication and expression.

Serology and Molecular Detection of Viral Infections – Hepatitis D

- The only member within the *Deltavirus* genus, HDV consists of a circular RNA genome and a single structural protein called hepatitis delta antigen within its core, surrounded by a viral envelope that is of HBV origin and contains the HBsAg.

Serology and Molecular Detection of Viral Infections – Hepatitis D

- Hepatitis D can either occur as a **co-infection** with hepatitis B, in which infection of HDV and HBV occurs simultaneously, or as a **superinfection**, in which HDV infects individuals who are already chronic HBV carriers.
- Clinically, co-infections usually resemble infection with HBV alone.

Serology and Molecular Detection of Viral Infections – Hepatitis D

- Superinfections result in a greater risk of developing fulminant hepatitis or chronic liver disease with an accelerated progression toward cirrhosis, liver decompensation, and hepatocellular carcinoma.
- Detection of hepatitis D utilizes molecular methods to detect HDV RNA, a marker of active viral replication that is present in all types of active hepatitis D infections.

Serology and Molecular Detection of Viral Infections – Hepatitis D

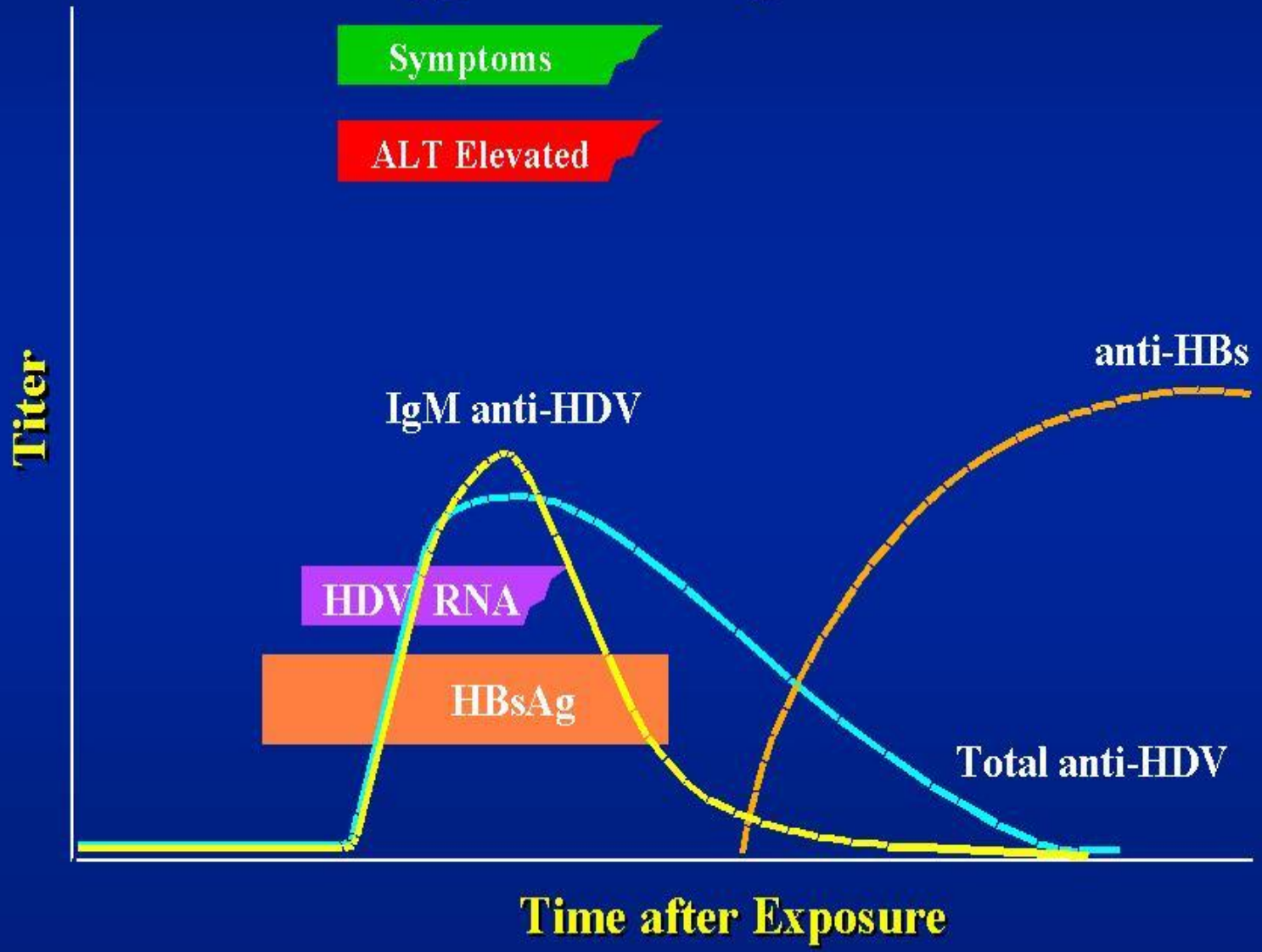
- HDV RNA is detected by reverse-transcriptase PCR assays, which are highly sensitive, specific, and quantitative.
- Hepatitis D infection is also indicated by the presence of anti-HDV in the patient's serum, detected by immunoassays employing hepatitis D antigen.

Serology and Molecular Detection of Viral Infections – Hepatitis D

- IgM anti-HDV may be used to detect acute hepatitis D.
- High titers of IgM and IgG antibodies are associated with chronic infection.

HBV - HDV Coinfection

Typical Serologic Course



Chronic HDV

- poor prognosis
- liver necrosis
- inflammation
- cirrhosis
- no effective anti viral drugs for chronic infection
- Vaccination against HBV provides immunity to HDV

Hepatitis E

- water borne hepatitis
- HEV- small, ss-RNA virus
- 4 genotypes
- Type 3 and 4 = pigs, wild boar and deers
- Fecal-oral route
- Ingestion of meat products, blood transfusion, vertical transmission
- 20 million cases annually in East and South Asia (WHO)

Hepatitis E

- Diagnosis Hepatitis E – detecting IgM anti-HEV
- RT-PCR
- Test for viral antigen detection

Hepatitis G

- HGV, aka GB virus-C (GBV-C)
- was first described in 1995-96
- ss RNA Flaviviridae
- 2-5% carrier rate
- persistent infection up to 9 years 15-30 trs adults
- often found as co infection with HBV, HCV and HIV

- possibility of not a true hepatitis virus
- MOT: same as HIV, HBV and HCV
- prevention: same as above
- no recommended treatment as of the moment

Serological Tests for Hepatitis

- Ouchterlony
- CIEP
- Rheophoresis
- CF Test
- Passive Agglutination
- Reverse Passive Agglutination
- RIA
- ELISA

Serological Tests for Hepatitis B

Screening Test

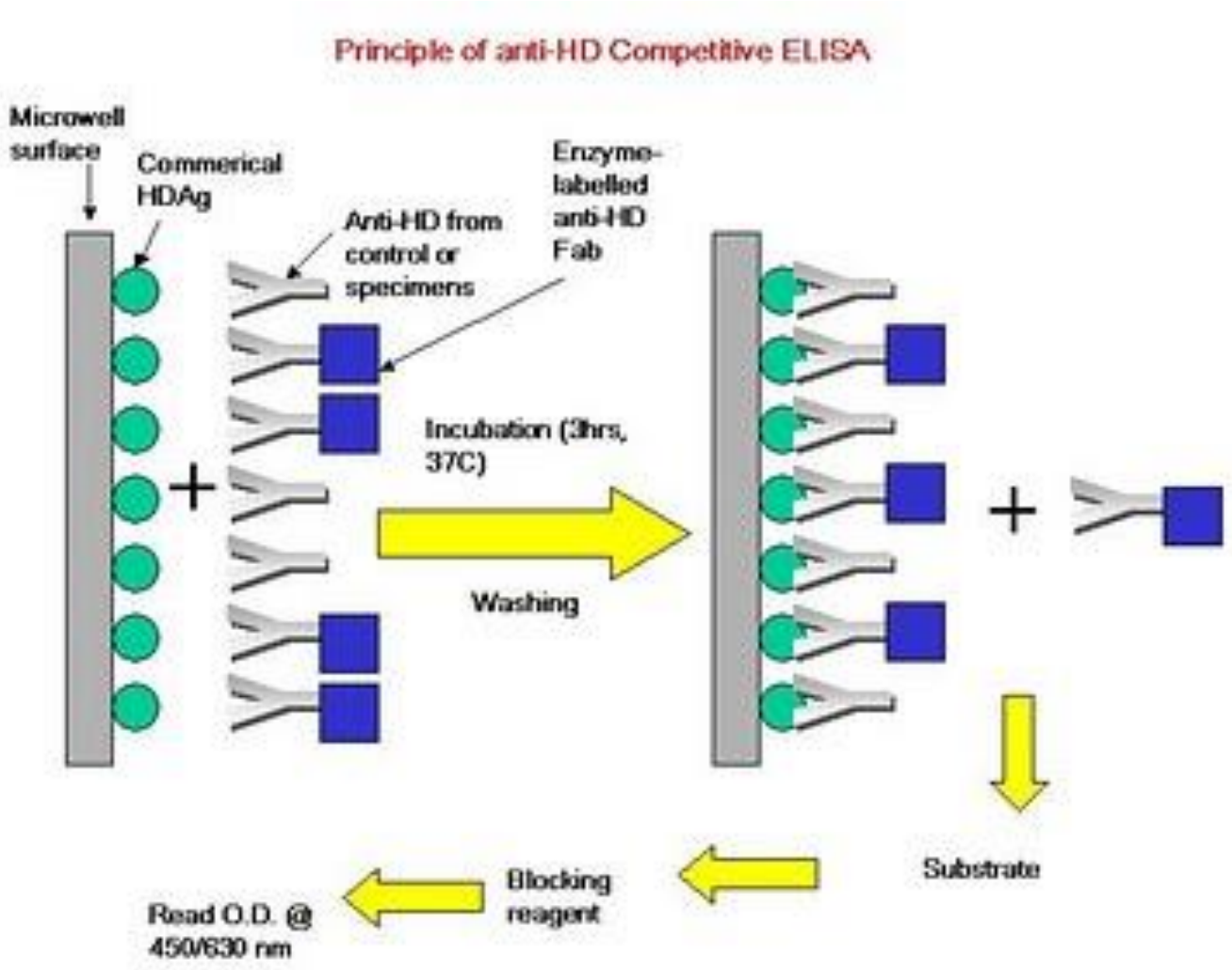
- Rapid Test
- Immunochromatographic assay
- The strip is coated with mouse monoclonal anti-Hbs
- If ab is present in serum, it binds with the antigen and moves along the strip chromatographically and forms visible line


HBsAg
MAX
MAX
MAX
MAX

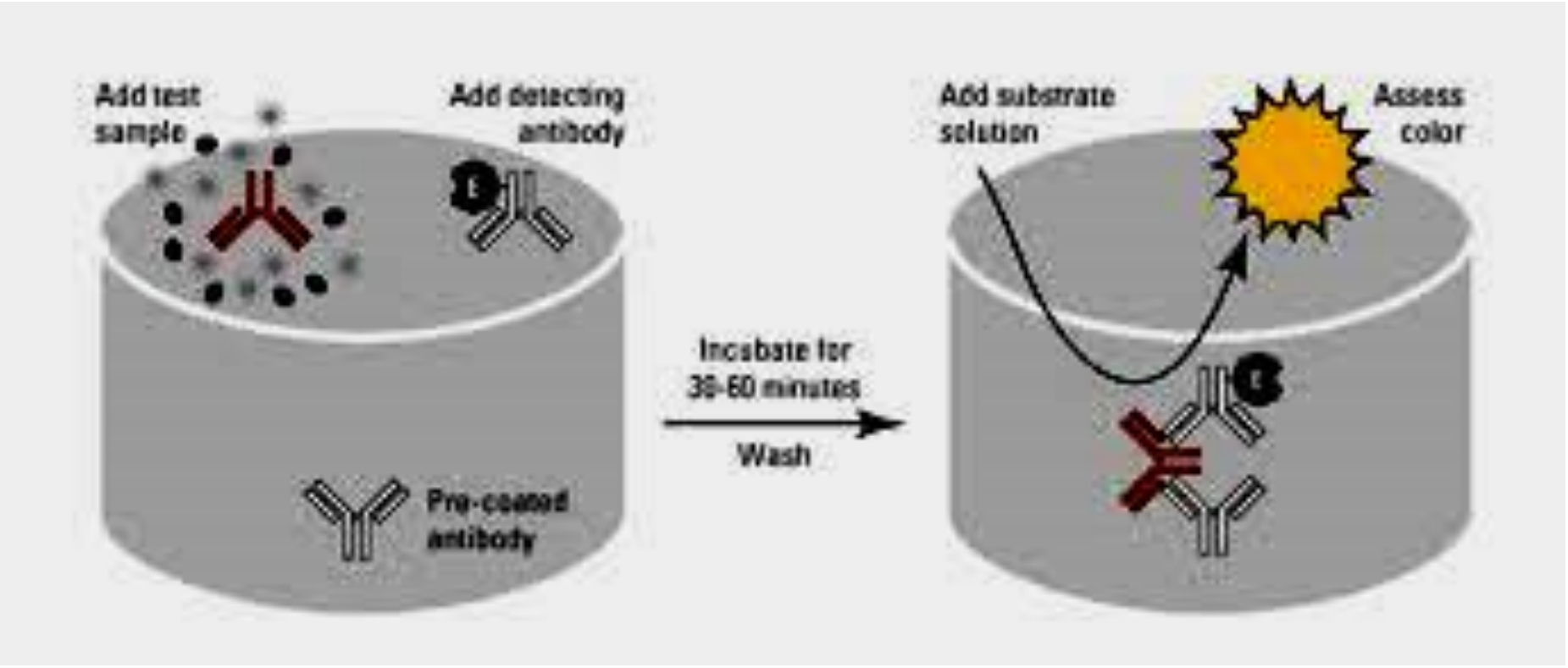
Screening Test ELISA

- Indirect ELISA: the OD value is directly proportional to antibody concentration

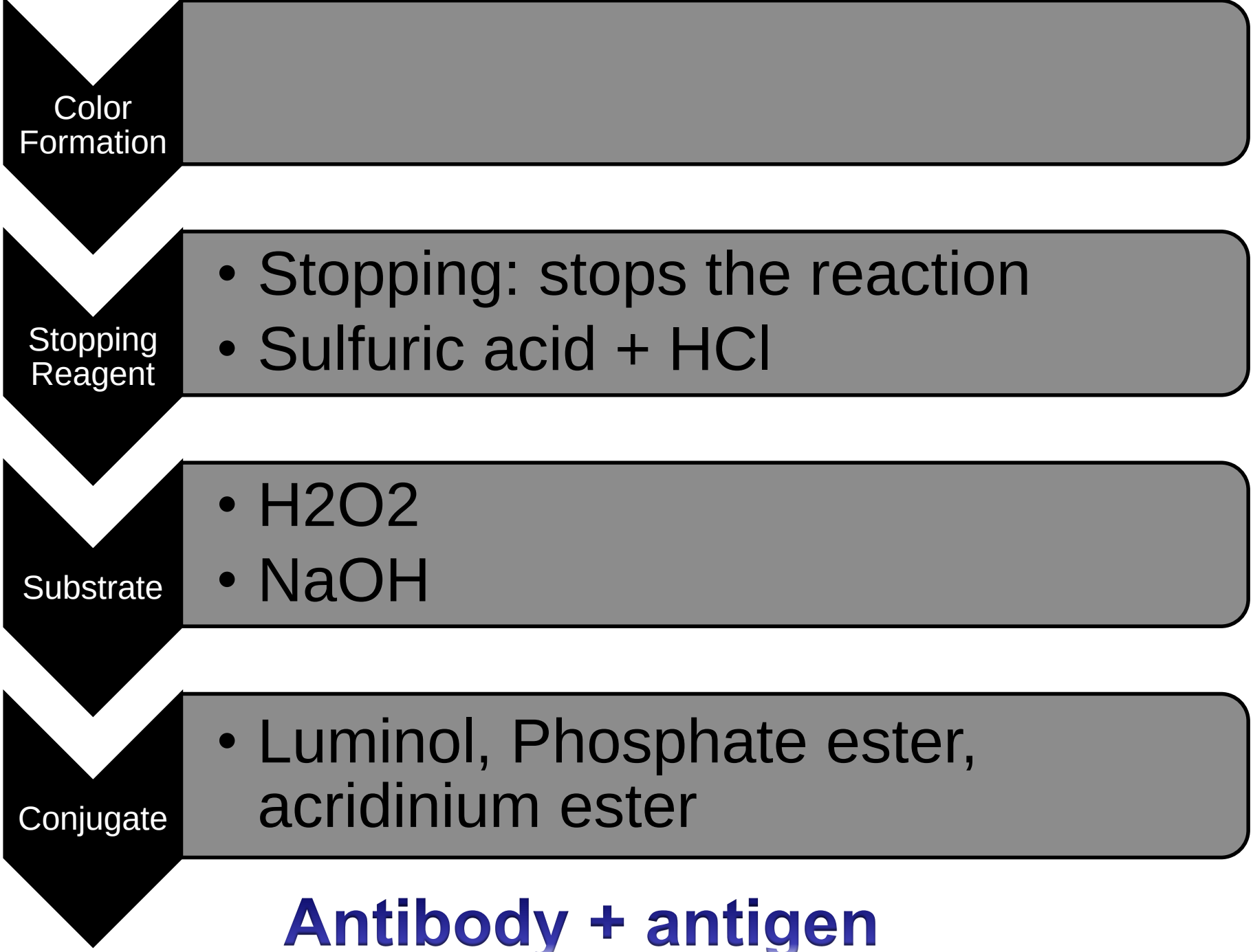
Competitive ELISA: requires less time to perform (sample and conjugate are added at the same time)



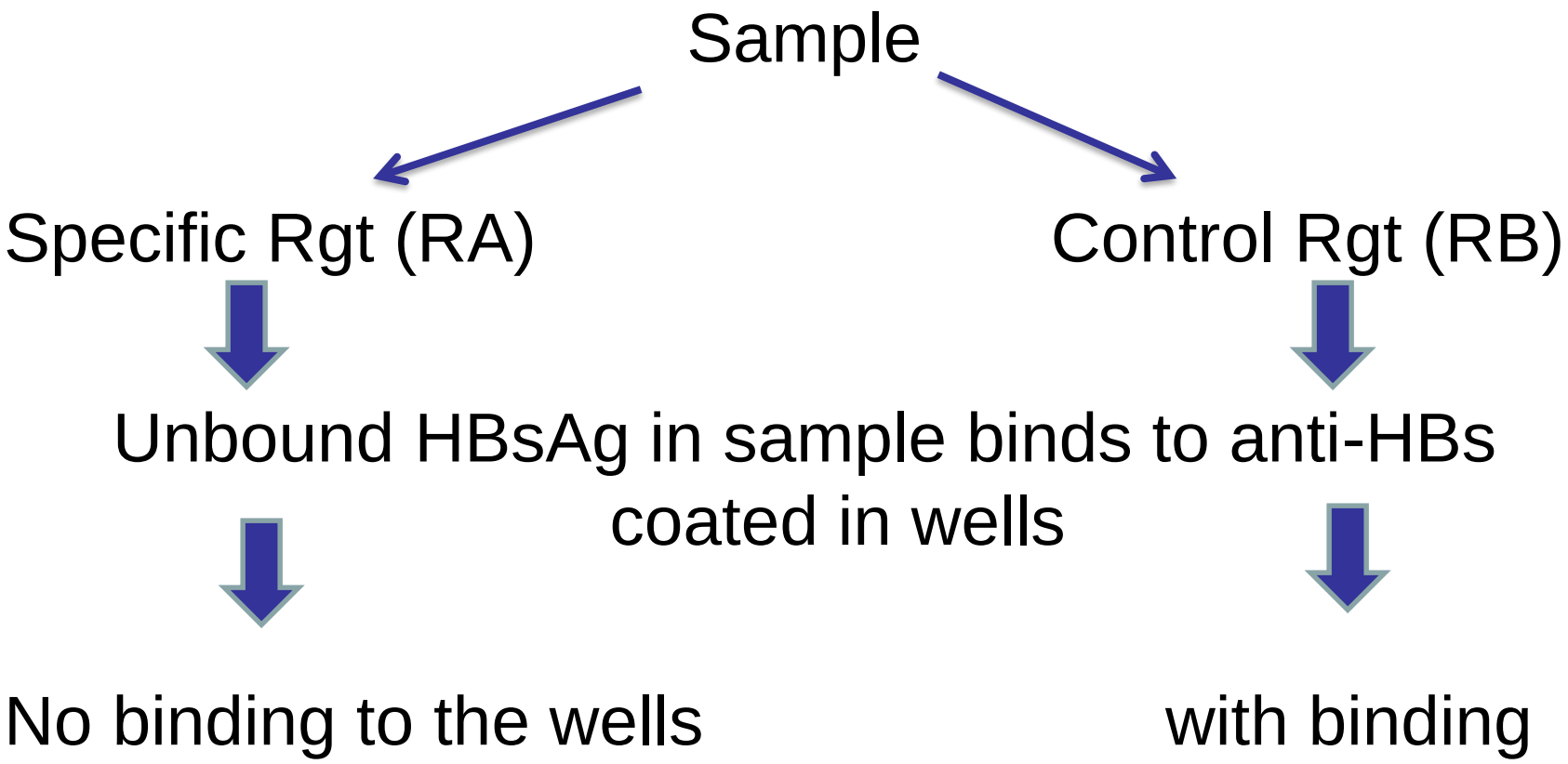
ELISA: Ag-Ab capture – targets a class of antibody. Other body fluids can be used aside from serum/plasma



Chemiluminiscent Assay Machine: used in serology, endocrinology, chemistry etc


Color Formation
Stopping Reagent
• Stopping: stops the reaction
• Sulfuric acid + HCl
Substrate
• H2O2
• NaOH
Conjugate
• Luminol, Phosphate ester, acridinium ester
Antibody + antigen

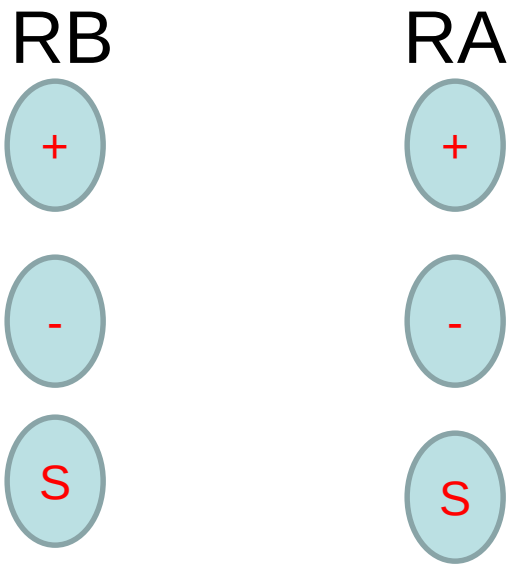
Confirmatory Assay: Neutralization assay-specific antibody



- Sample is confirmed + if the inhibition in the specific wells exceeds 50% and the reading in the control reagent is the cut off value

HBsAg Confirmatory Test

Principle of the Test



The assay is run in according to usual screening .

Procedure: Two wells are well aligned to each sample. The specific reagent will compete with the mouse antibody. Coated in the wells for any HBsAg present in the sample and will reduce the amount of HBsAg binding in the well. In the control reagent there is no competition and it will bind normally

Interpretation

Sample Reactivity Ratio	Inhibition Percentage	Interpretation
> or equal 0.8	>50%	Positive
< 0.8	Whatever results	Negative
>0.8	<50%	For dilution

Note: For strongly reactive samples, if the inhibition is less than 50% and the absorbance in the control reagent 3,500…the sample should be diluted with 1:100 or 1:10,000

Tests	Cutoff Value	Patient's Value	Interpretation
HBsAg	1.00	0.52	Non-Reactive
Anti-HBs	10.0	>1000.00	Reactive
Anti-HBe	1.00	3.16	Non-Reactive

For anti-HBcIgG, anti-HBe and anti-HAVIgG, results below the cut off value are considered as REACTIVE.

Monitoring Test

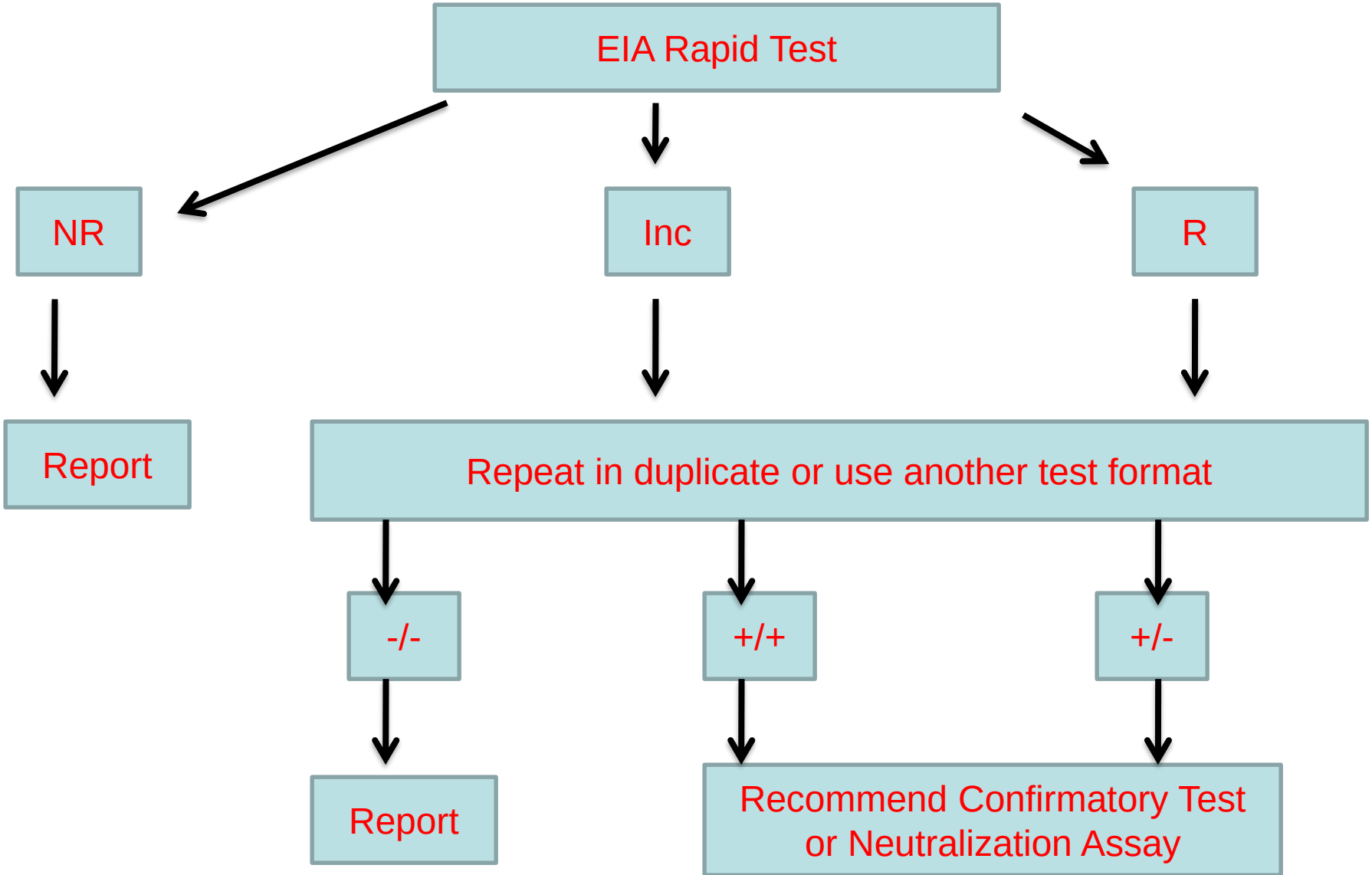
Branched DNA Assay

- Principle: sandwich nucleic acid hybridization.
- For research purposes only

PCR

- Principle: DNA amplification

HBsAg Testing for Algorithm

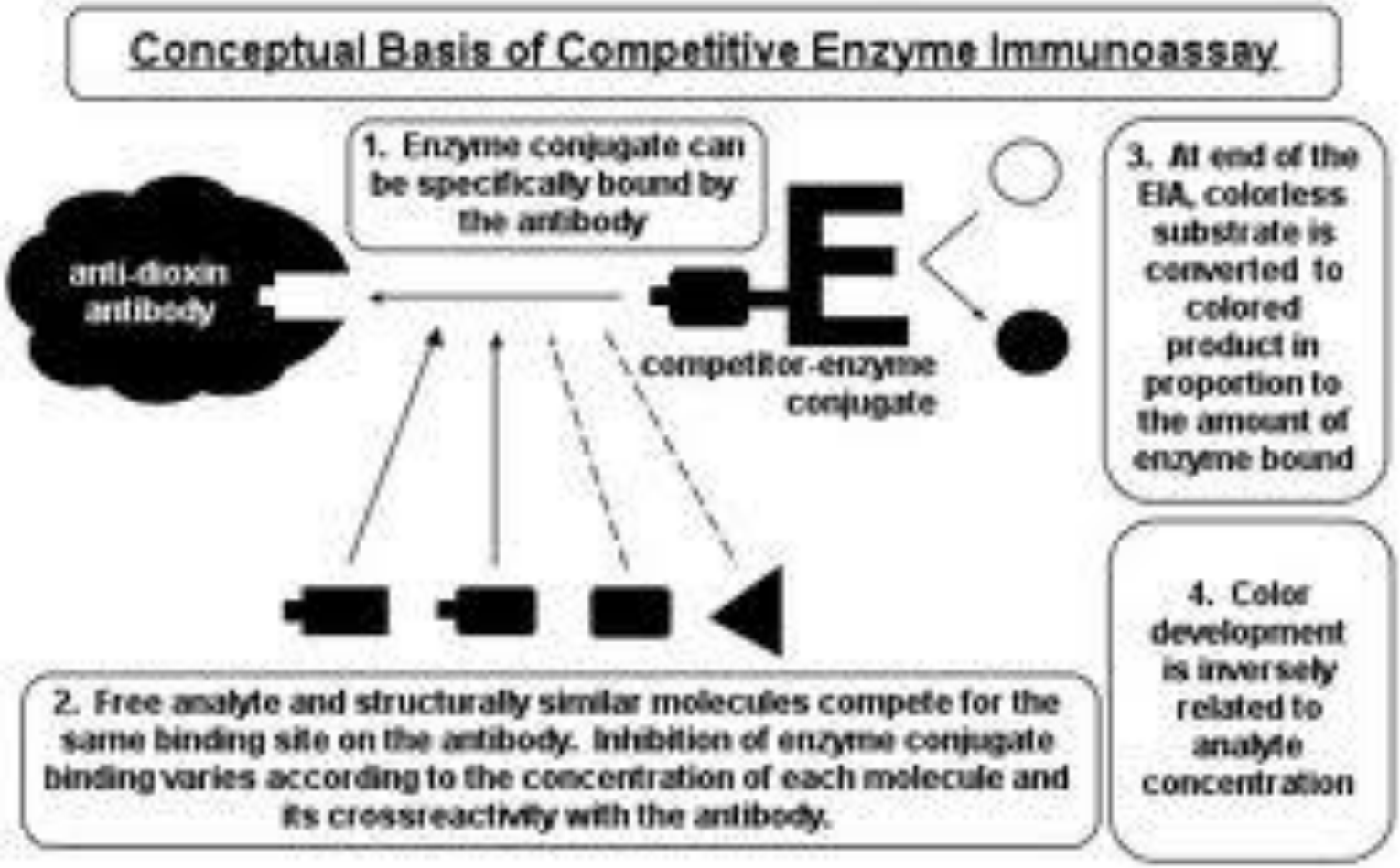


Serological Test for Hepatitis C

Available Test

Types	Examples
Screening	EIA Rapid Tests
Confirmatory/ Supplemental	SIA (e.g. RIBA, LIA) HCV RNA (Qualitative)
Monitoring	HCV RNA (Quantitative)
Other Tests	Genotyping

EIA Screening Test for HCV



Supplemental/Confirmatory Assay

- To establish true positivity of anti-HCV EIA
- Higher specificity but lower sensitivity

	Gold Std (+)	Gold Std (-)
Test (+)	True Positive	False Positive
Test (-)	False Negative	True Negative

RIBA

In most cases, it can tell if the positive anti-HCV test was due to exposure to HCV (positive RIBA) or represents a false signal (negative RIBA). The RIBA test cannot distinguish between a current or past infection.

HCV RNA Qualitative

- may be used to distinguish between a current or past infection.
- It is reported as a "positive" or "detected" if any HCV viral RNA is found; otherwise, the report will be "negative" or not detected."
- It may also be ordered after HCV treatment is complete to see if the virus has been eliminated from the blood.
- These tests are seldom used any more.

Interpretation of Strip Immunoblot Assay

- No band visible: negative for anti-HCV
- At least 2 bands: + for anti-HCV
- One band: indeterminate

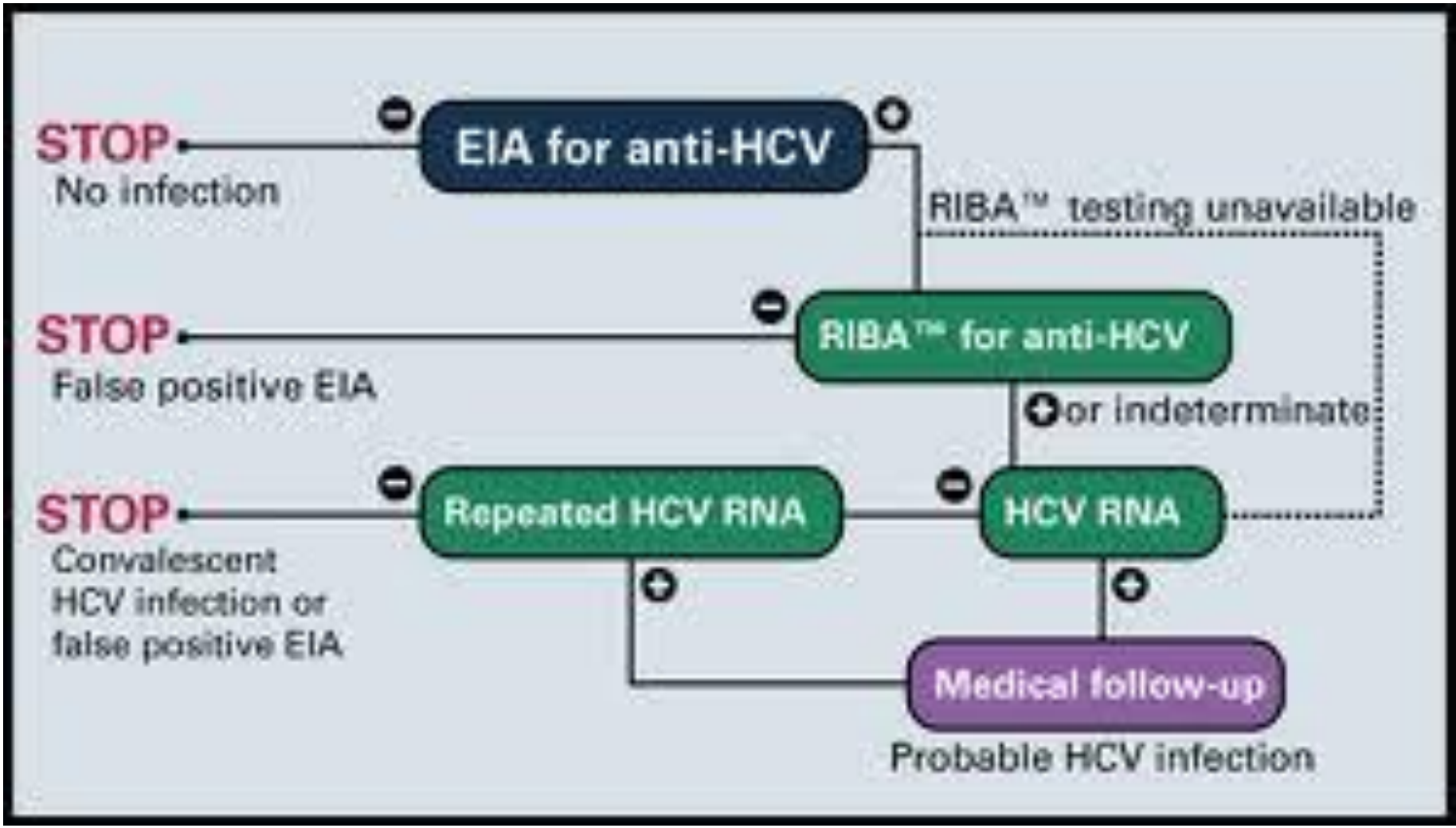
Anti-HCV IgM Assay

- Acute infection 50-93%
- Chronic infection 50-70%

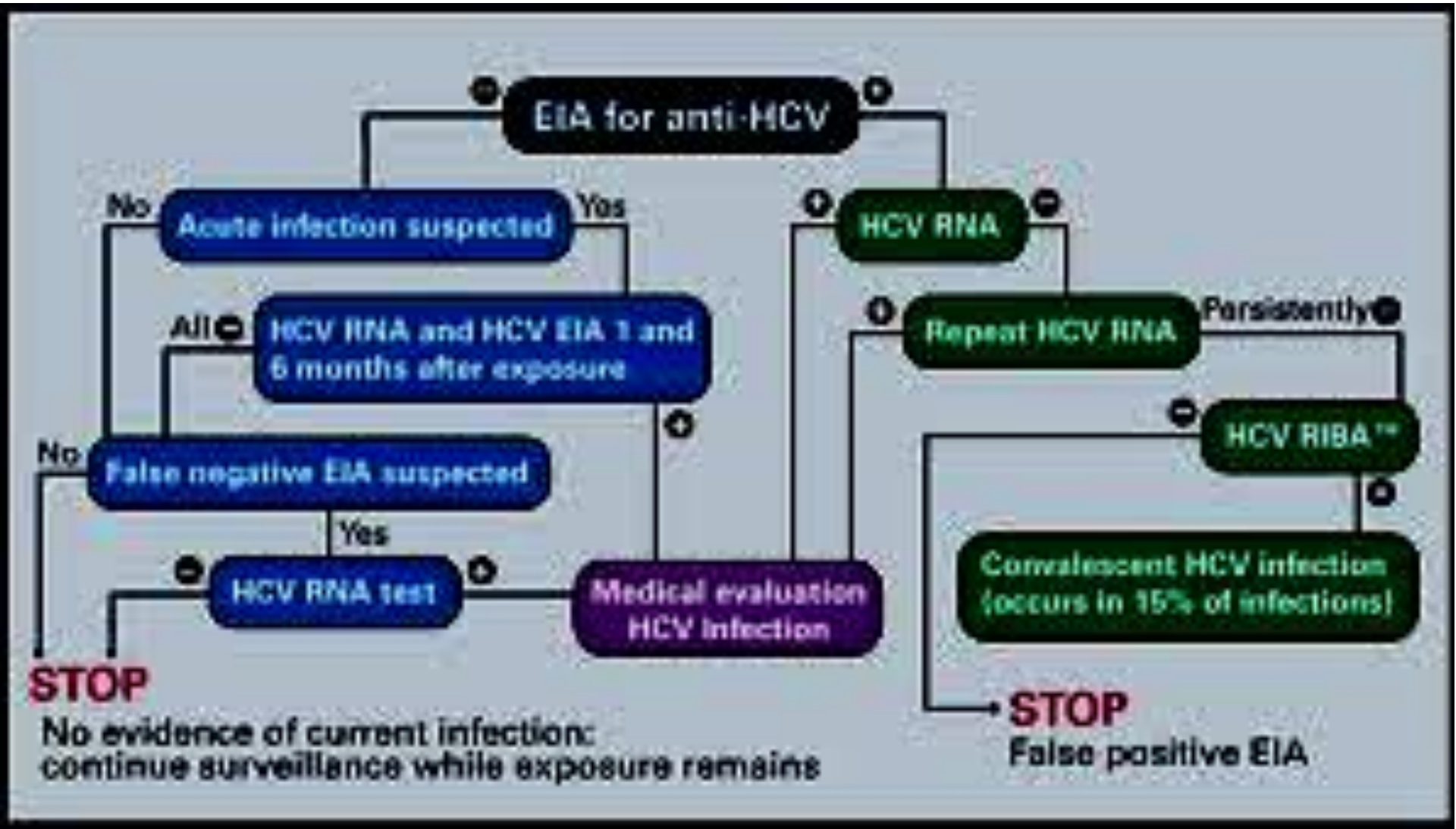
- **HCV Viral Load (HCV RNA test, Quantitative)** detects and measures the number of viral RNA particles in the blood. Viral load tests are often used before and during treatment to help determine response to treatment by comparing the amount of virus before and during treatment (usually at several time points in the first three months of treatment). Successful treatment causes a decrease of 99% or more (2 logs) in viral load soon after starting treatment (as early as 4-12 weeks) and usually leads to viral load being not detected even after treatment is completed. Some newer viral load tests can detect very low amounts of viral RNA.

- **Viral genotyping** is used to determine the kind, or genotype, of the HCV virus present. There are 6 major types of HCV; the most common (genotype 1) is less likely to respond to treatment than genotypes 2 or 3 and usually requires longer therapy (48 weeks versus 24 weeks for genotype 2 or 3). Genotyping is often ordered before treatment is started to give an idea of the likelihood of success and how long treatment may be needed.

Algorithm for HCV



Interpretation of the Test



Thank you for listening!!

Sawadee krup!!