SNS COLLEGE OF TECHNOLOGY

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Department of Biomedical Engineering

Course Name: 19GET277 – Biology for Engineers

IV Year : VII Semester

UNIT III – HUMAN DISEASES

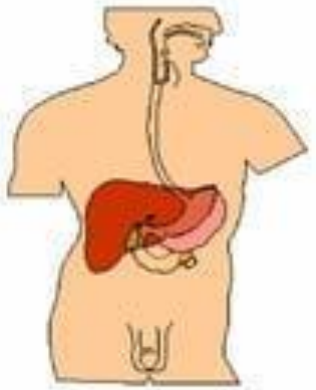
Topic : Definition, Causes, symptoms, Diagnosis, Treatment and prevention of Hepatitis

What is Viral Hepatitis

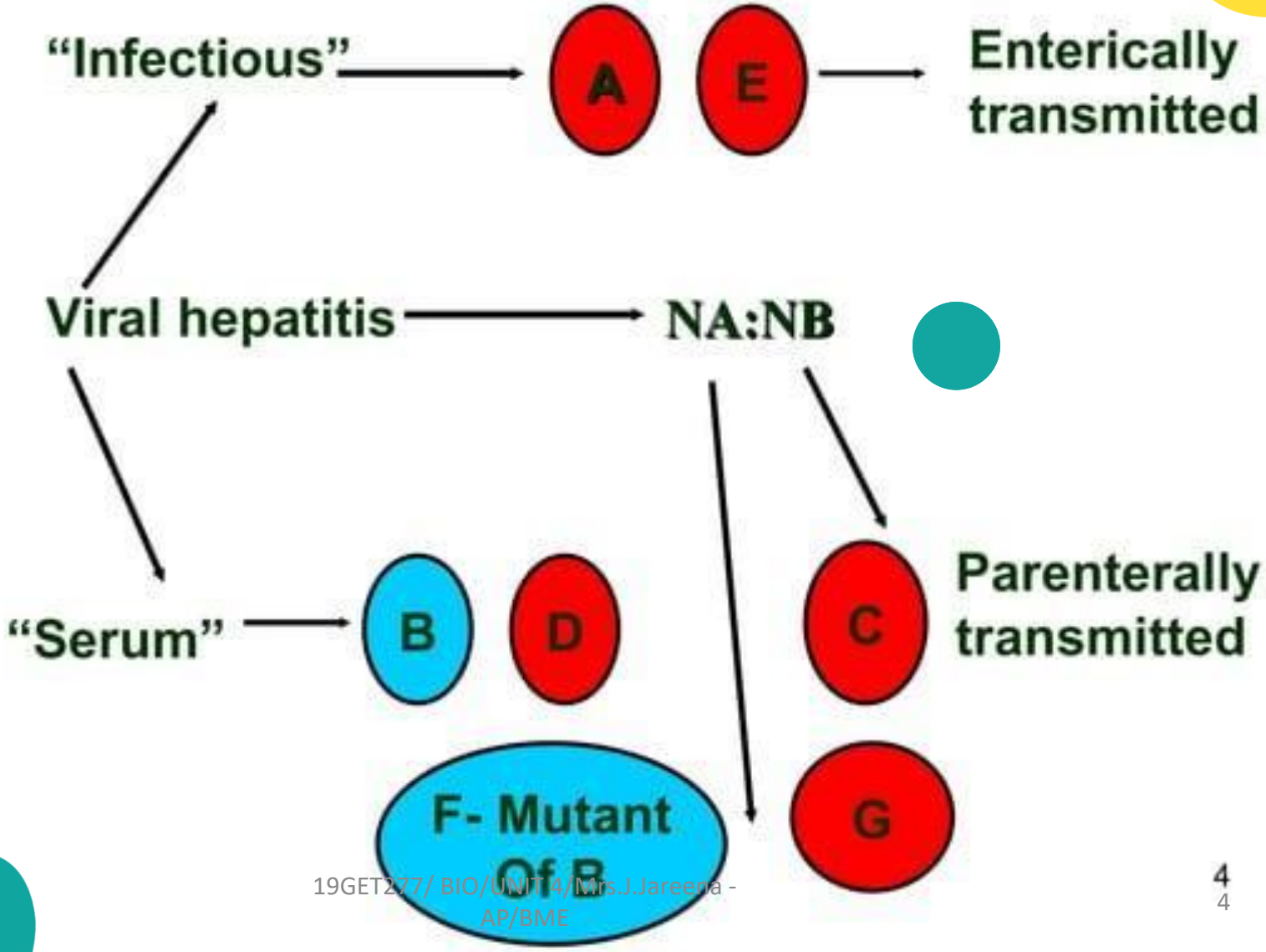
- Viral hepatitis is a systemic disease with primary inflammation of the liver by any one of heterogenous group of hepatotropic viruses

HEPATITIS VIRUSE

- Hepatitis A (HAV) Picornaviridae (1973)
- Hepatitis B (HBV) Hepadnaviridae (1970)
- Hepatitis C (HCV) Flaviviridae (1988)
- Hepatitis D (HDV) ? (1977)
- Hepatitis E (HEV) (Caliciviridae) (1983), Hepeviridae
- Hepatitis F – Not separate entity – Mutant of B Virus.
- Hepatitis G (HGV) Flaviviridae (1995)



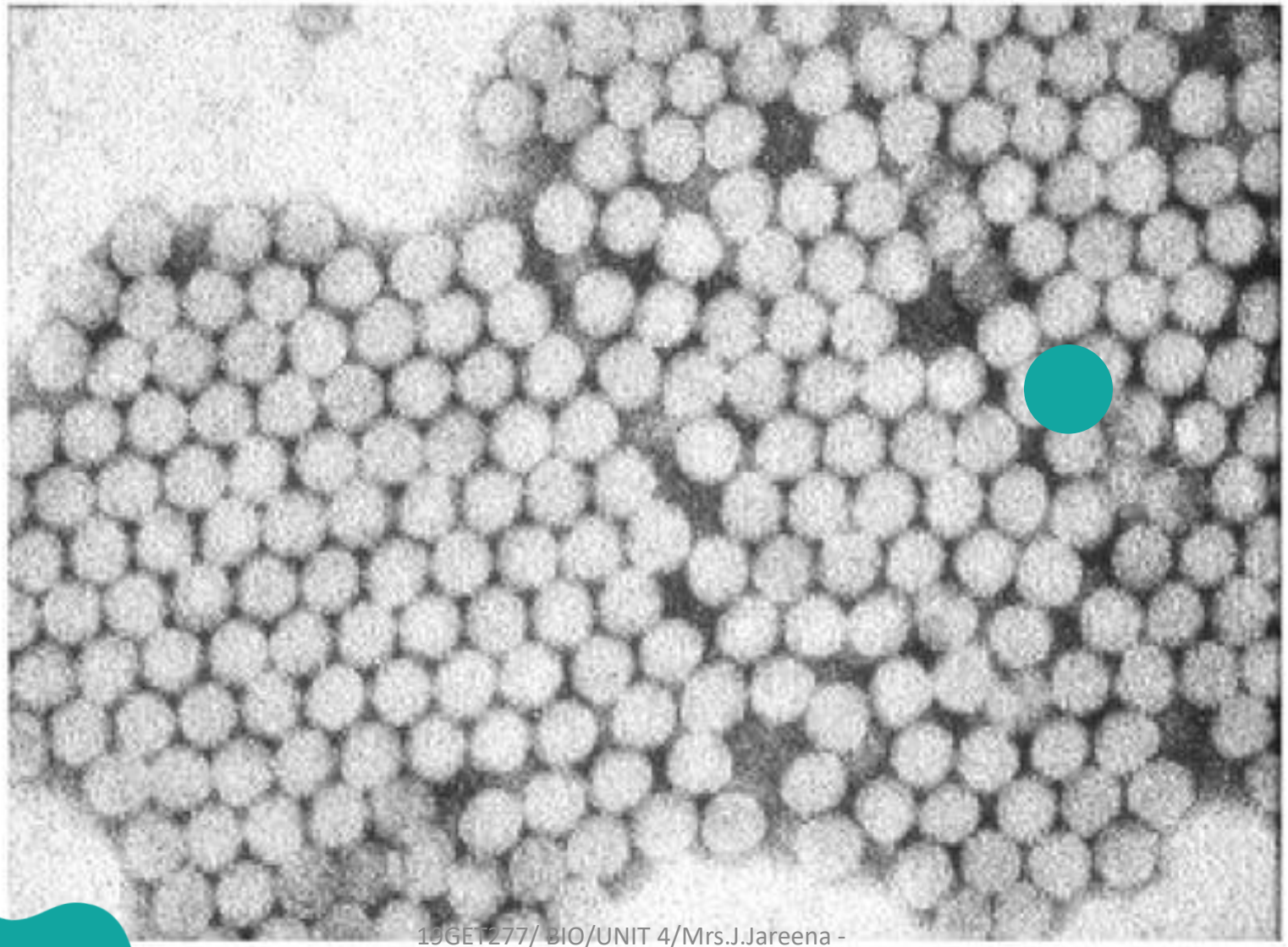
Viral Hepatitis - Histo Perspectives



Type of Hepatitis

	A	B	C	D	E
Source of virus	Feces	Blood Blood derived Body fluids	Blood Blood derived Body fluids	Blood Blood derived Body fluids	Feces
Route of Transmission	Feco-oral	Percutaneous Permucosal	Percutaneous Permucosal	Percutaneous Permucosal	Feco-oral
Chronic Infection	No	Yes	Yes	Yes	No
Prevention	Pre Post Exposure Immunization	Pre Post Exposure Immunization Blood donor screening	Blood donor screening	Pre Post Exposure Immunization	Ensure Safe Drinking water

HAV



Hepatitis A Virus

Naked RNA virus

Related to enteroviruses, formerly known as Enterovirus 72, now put in its own family: heptoviridae

One stable serotype only

Difficult to grow in cell culture: primary marmoset cell culture and also in vivo in chimpanzees and marmosets

4 genotypes exist, but in practice most of them are group 1

RESISTANCE (HAV

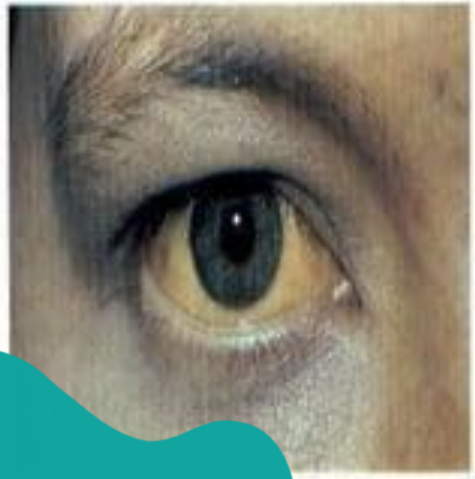
- Resistant to inactivation by heat at 60^0 C for one hour, ether & acid at pH 3.
- Inactivated by boiling for one minute, 1: 4,000 formaldehyde at 37^0 C for 72 hours & chlorine 1 ppm for 30 minutes.
- Not affected by anionic detergents.
- Survives prolonged storage at 4^0 C or below.

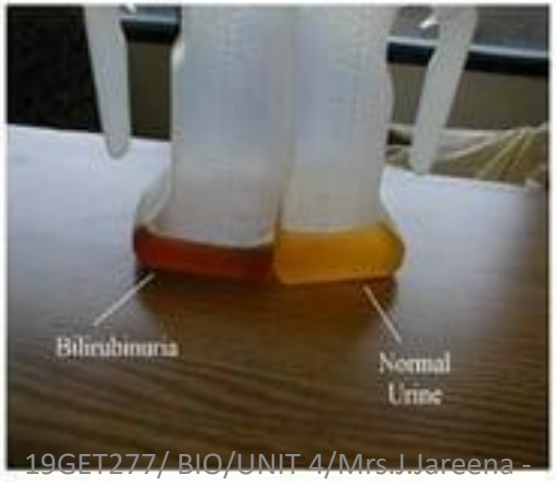
Hepatitis A Virus Transmission

- Close personal contact
  (e.g., household contact, sex contact, child day care centers)
- Contaminated food, water
  (e.g., infected food handlers, raw shellfish)
- Blood exposure (rare)
  (e.g., injecting drug use, transfusion)

romal or Preicteric phase : (symptoms: fatigue, joint- and abdominal pain, malaise, vomiting, lack of appetite, hepatomegaly)

Icteric phase: Icterus: jaundice (skin, sclera, mucous membranes, cause: elevated bilirubin level, bilirubinuria: dark urine, pale stool)





PATHOGENESIS - HA

Cause subacute disease in children & young adults.

HAV invade into human body by fecal-oral route, multiplies in the intestinal epithelium & reaches the liver by hematogenous spread.

After one week, the HAV reach liver cells replicate within.Then enter intestine with bile and appear in feces.

Incubation Period : 2 to 6 weeks.

PATHOGENESIS – HAV contd--

- After HAV replicating and discharging,liver cells damage begin
- Animal experiment proved that immune complex may attend the pathogenesis of HAV
- Complement level reduce the pathogenesis maybe following:
- activated T cell secrete γ-INF that promote the representation of HLA on the liver cells,CTL may kill the target cell infected with HAV

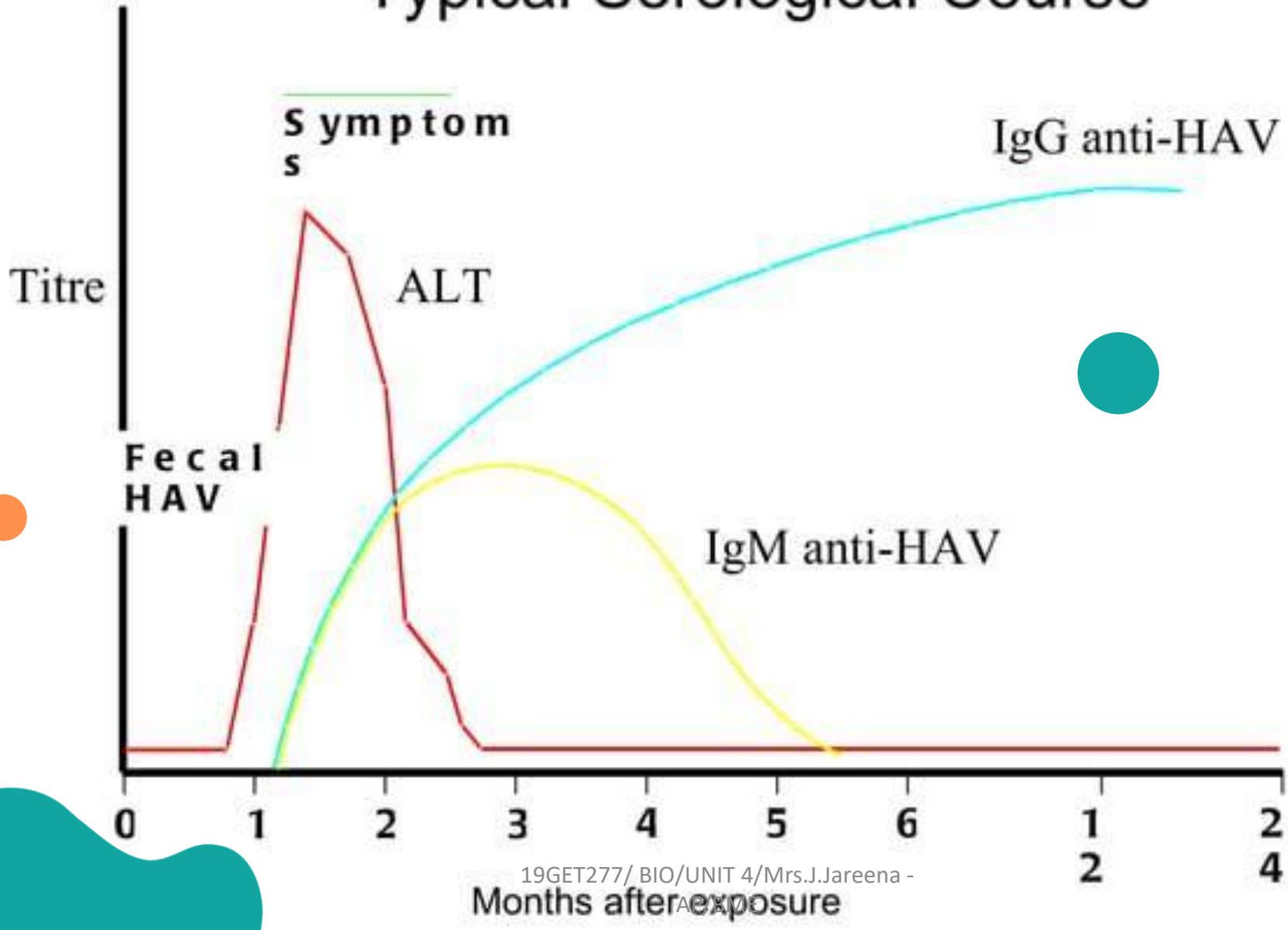
LAB.DIAGNOSIS

1. Demonstration of Virus in feces:
   By: Immunoelectron microscopy

4. Virus Isolation:
5. Detection of Antibody :By ELISA
4. Biochemical tests:
   i) Alanine aminotransferase (ALT)
   ii) Bilirubin
   iii) Protein
5. Molecular Diagnosis : RT PCR of feces

Hepatitis A Infection

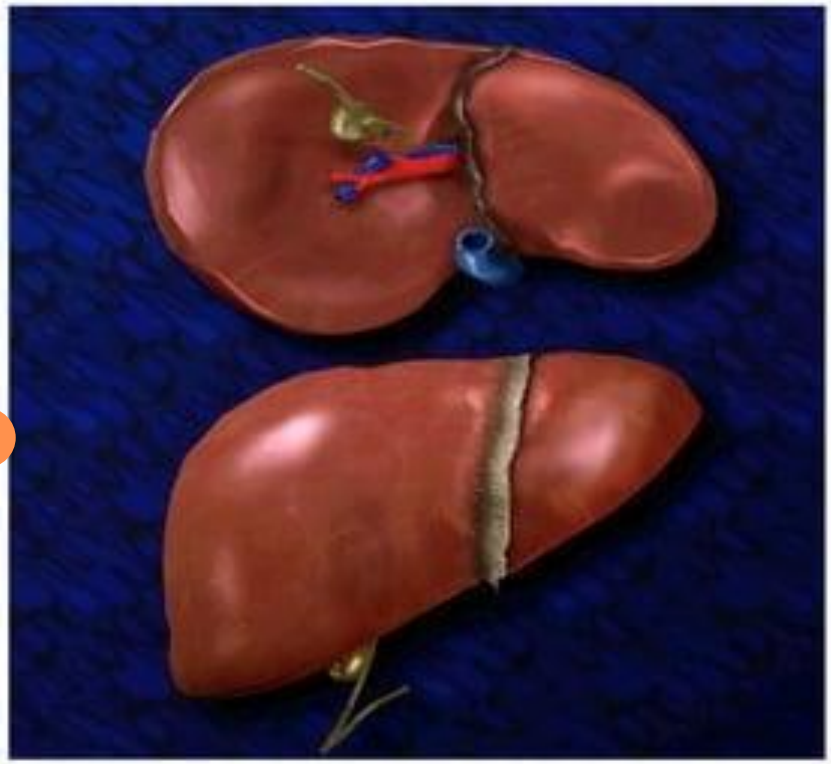
Typical Serological Course

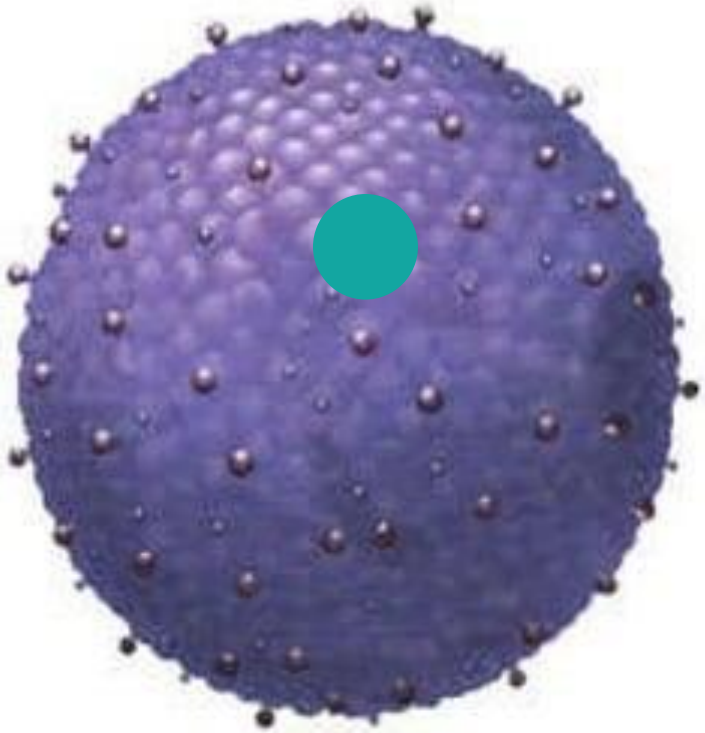


Hepatitis A Vaccination Strategi
Epidemiologic Considerations

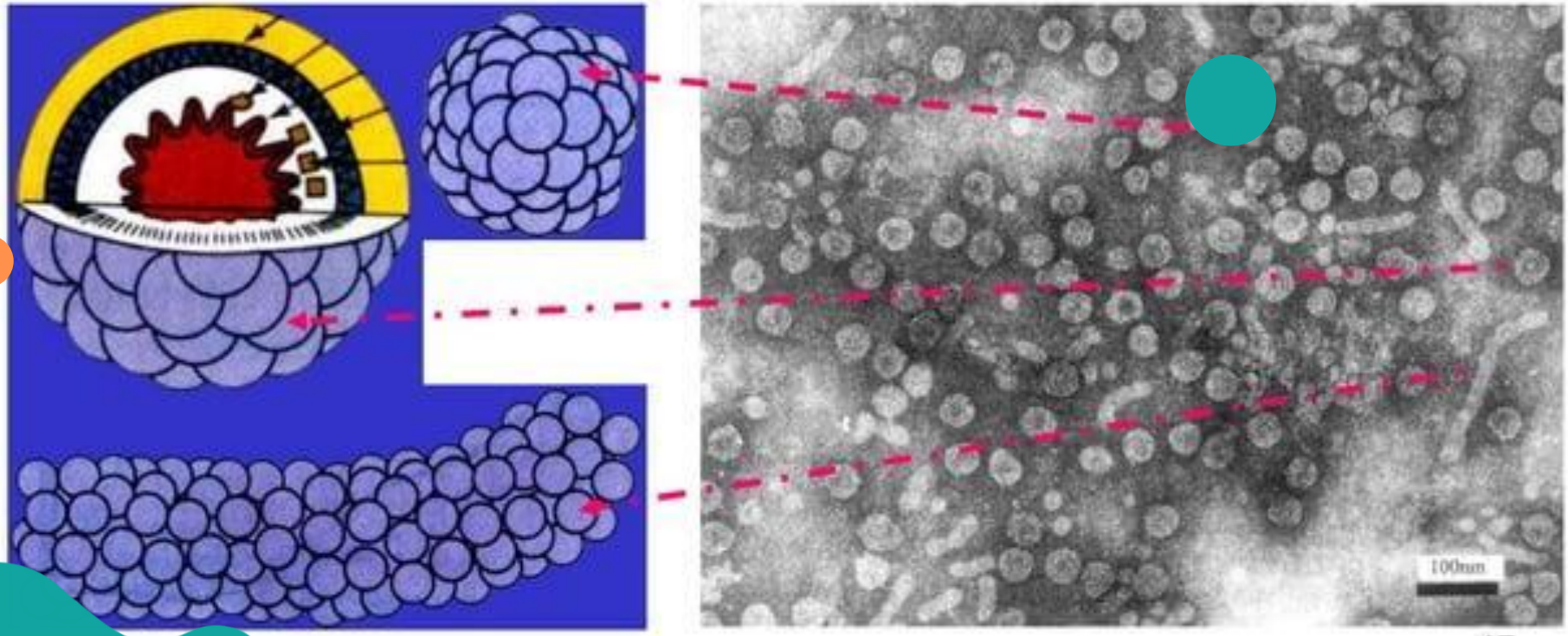
- Many cases occur in community-wide outbreaks
  - no risk factor identified for most cases
  - highest attack rates in 5-14 year olds
  - children serve as reservoir of infecti
- Persons at increased risk of infection
  - travelers
  - homosexual men
  - injecting drug users

Hepatitis B Virus





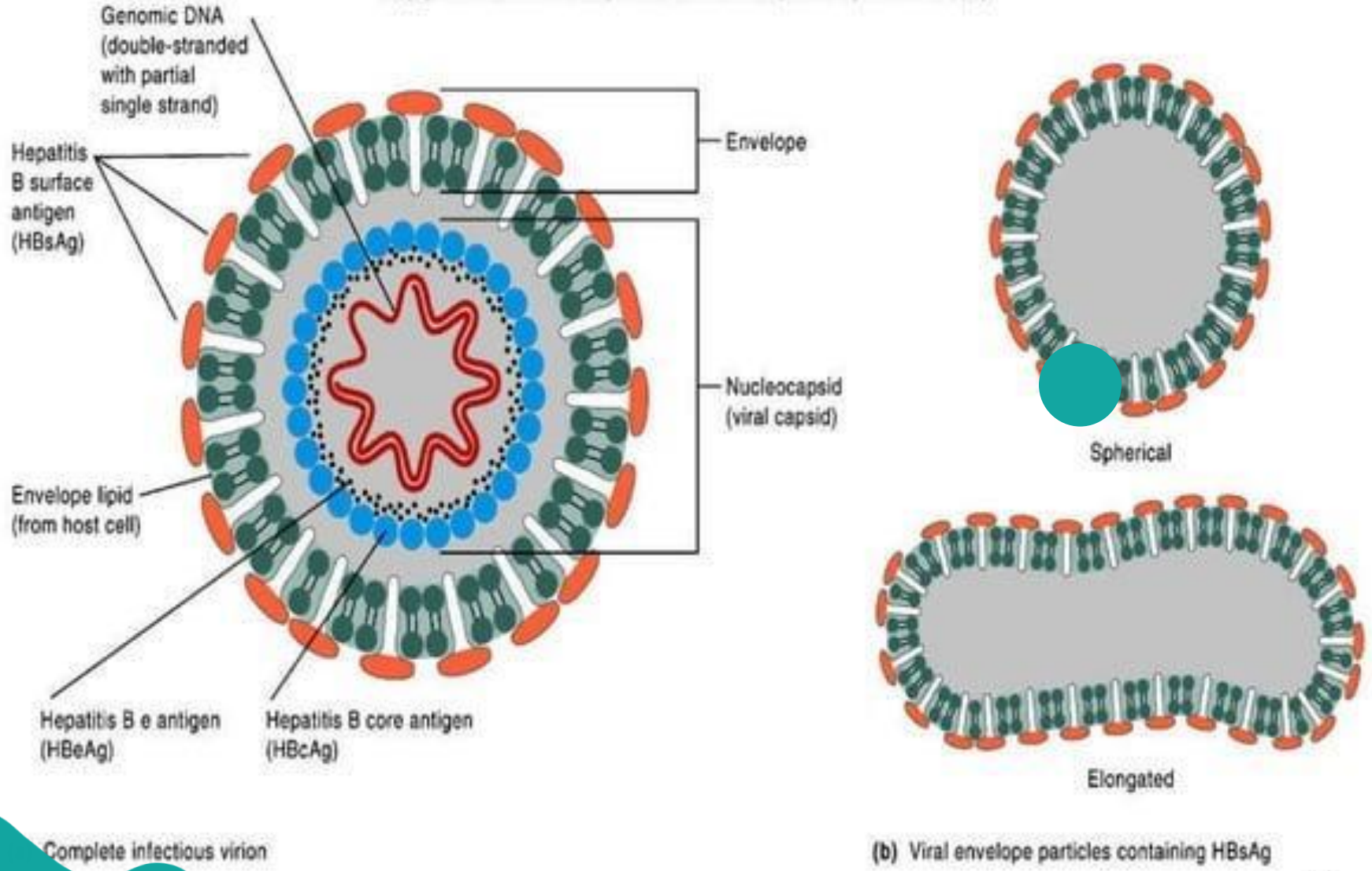
Hepatitis B Virus



1、 Properties of HBV

- a member of the hepadnavirus group
- Circular partially double-stranded DNA viruses
- Replication involves a reverse transcriptase.
- endemic in the human population and hyperendemic in many parts of the world.
- a number of variants
- It has not yet been possible to propogate the virus in cell culture

HBV : Structure

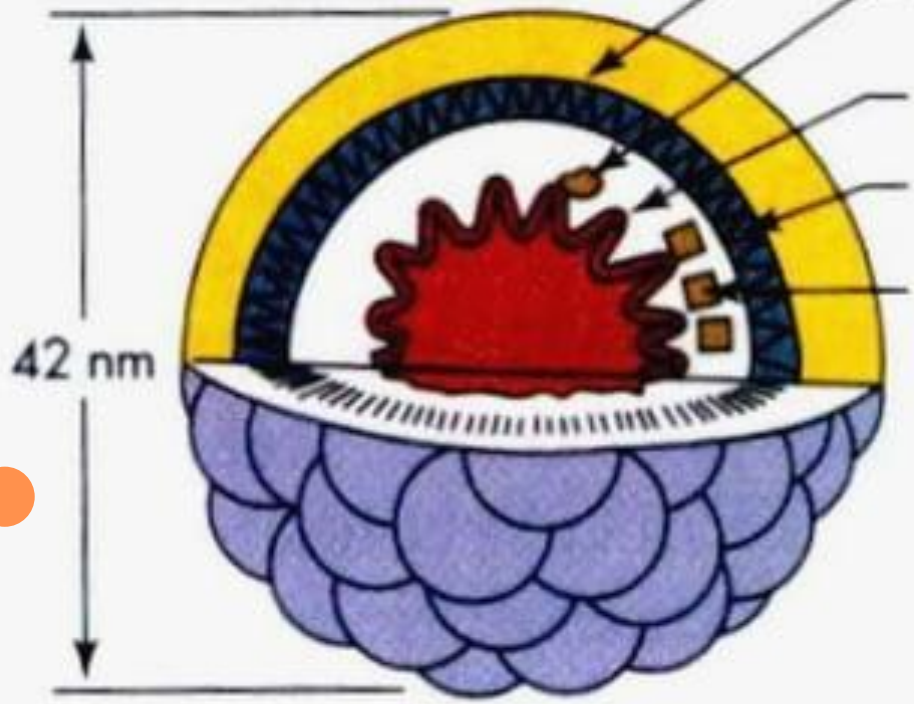


HBV : Structure

- Virion also referred to as Dane particle (ds-tranded DNA)
- 42nm enveloped virus
- Core antigens located in the center (nucleocapsid)
  * Core antigen (HBcAg)
  * e antigen (HBeAg)- an indicator of transmissibility (minor component of the core- antigenically distinct from HBcAg)
- 22nm spheres and filaments other forms- no DNA in these forms so they are not infectious (composed of surface antigen)- these forms outnumber the actual virions

HBV Structure & Antigens

Dane particle



L
M
S

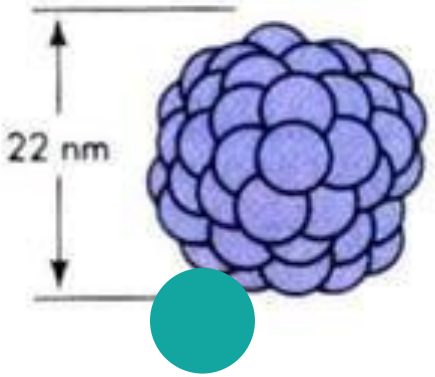
HBsAg = surface (coat) protein (**4 phenotypes : adw, adr, ayw and ayr**)

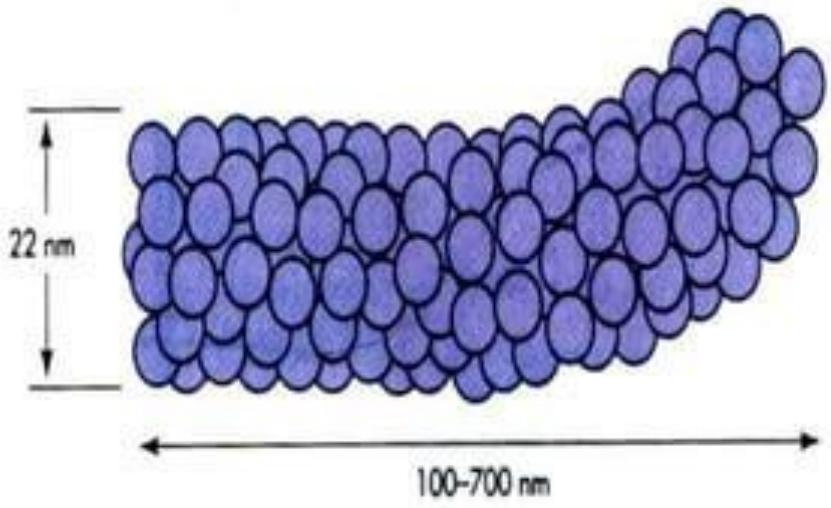
HBcAg = inner core protein (**a single serotype**)

HBeAg = secreted protein, function unknown

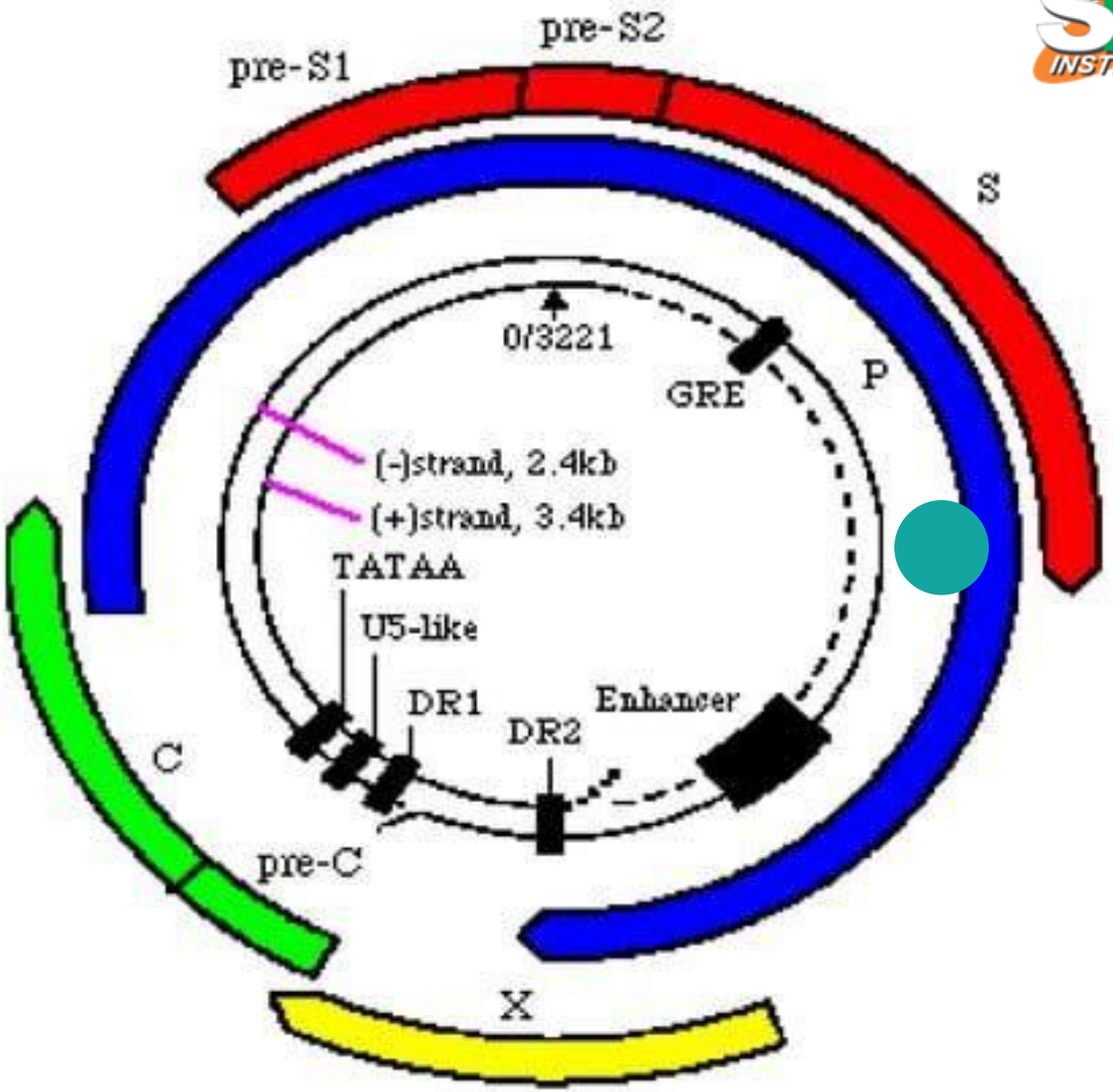
decoy particles

- HBsAg-containing particles are released into the serum of infected people and outnumber the actual virions.
- Spherical or filamentous
- They are immunogenic and were processed into the first commercial vaccine against HBV.





GENOME

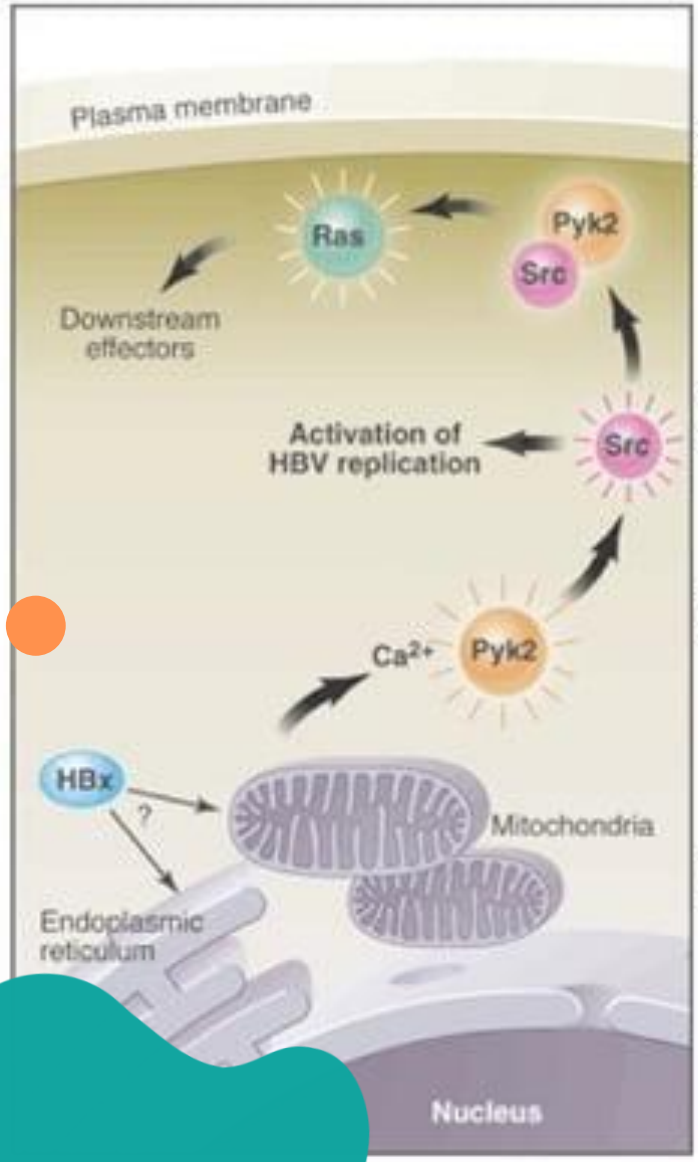

pre-S2
pre-S1
S
0/3221
GRE
P
(-)strand, 2.4kb
(+)strand, 3.4kb
TATAA
U5-like
DR1
DR2
Enhancer
C
pre-C
X

Open Reading Frames

There are 4 open reading frames derived from the same strand (the incomplete + strand)

- **S** - the 3 polypeptides of the surface antigen (**preS1, preS2 and S** - produced from alternative translation start sites.
- **C** - the core protein
- **P** - the polymerase
- **X** - a transactivator of viral transcription (and cellular genes?). HBx is conserved in all mammalian (but not avian) hepadnaviruses. Though not essential in transfected cells, it is required for infection in vivo.

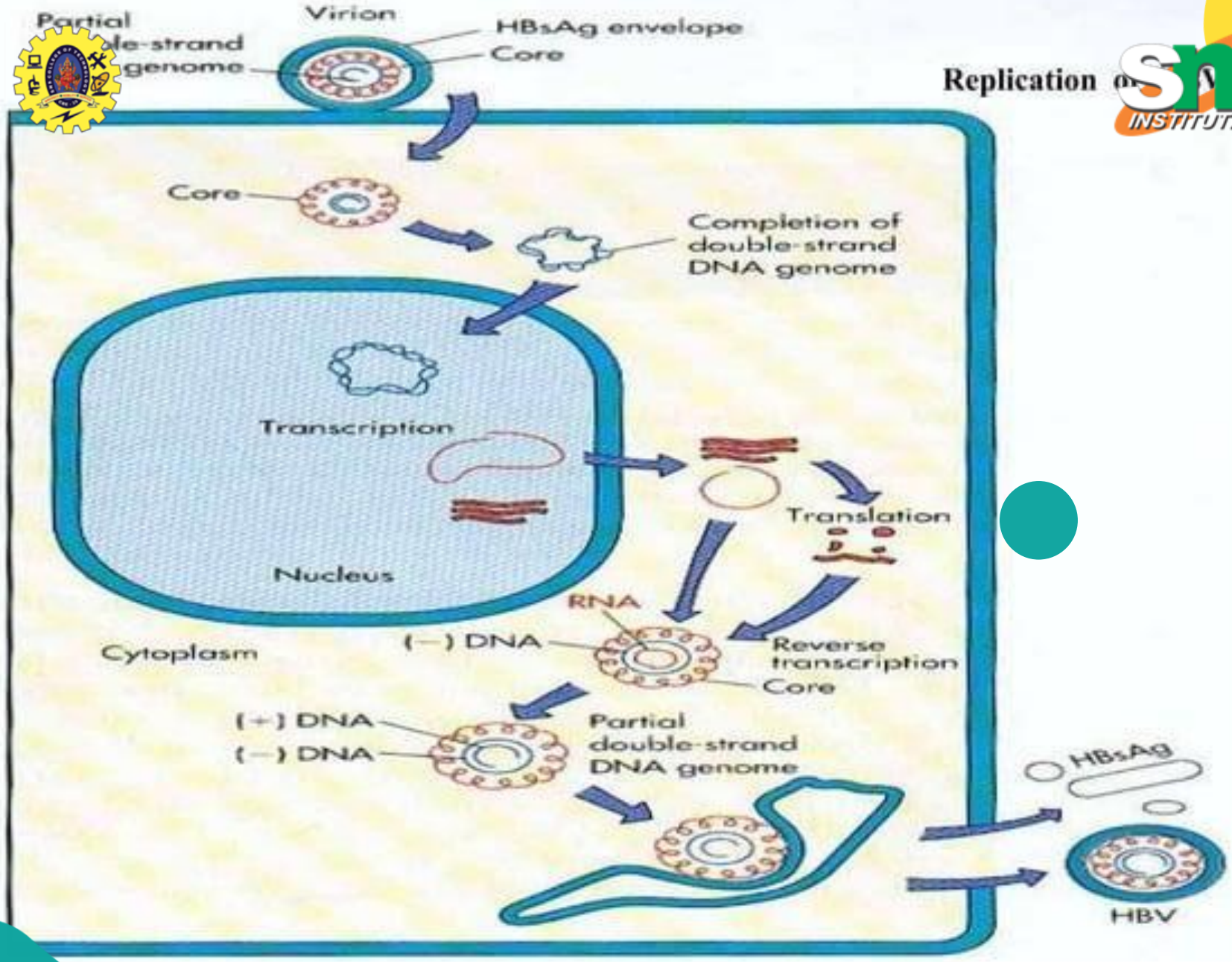
HBx activates the Ras-Raf-MAPK cascade. Influence on cell proliferation?



- Unlike the magnitude of Ras activation by growth factors, HBx stimulation is modest but sustained
- Downstream signaling: activate transcription factors (eg. AP-1, NF-KB and c-myc)
- Activation of the Ras-Raf-MAPK signaling pathway is essential for HBx activation of AP-1 and NF-KB
- HBx-mediated activation of the Ras-Raf-MAPK pathway has been linked to accelerated entry of cells into S phase

2、 HBV: Replication

- Reverse transcription: one of the mRNAs is replicated with a reverse transcriptase making the DNA that will eventually be the core of the progeny virion
- RNA intermediate: HBV replicates through an RNA intermediate and produces and release antigenic decoy particles.
- Integration: Some DNA integrates into host genome causing carrier state


Partial
le-strand
genome
Virion
HBsAg envelope
Core
Replication
Core
Completion of
double-strand
DNA genome
Transcription
Nucleus
Cytoplasm
Translation
RNA
(−) DNA
Reverse
transcription
Core
(+) DNA
(−) DNA
Partial
double-strand
DNA genome
HBsAg
HBV

3、 HBV: Modes of Transmission

- Parenteral - IV drug abusers, health workers are at increased risk.
- Sexual - sex workers and homosexuals are particular at risk.
- Perinatal (**Vertical**) – mother (HBeAg+) →infant.

4、 Epidemiology

- 350,000,000 carriers worldwide
- 120,000,000 carriers in China

  - the carrier rate can exceed 10%

  -15 to 25% of chronically infected patie will die from chronic liver disease
- 500,000 deaths/year in China
- 982,297 liver disease in China 2005
- 50% of children born to mothers with chronic HBV in the US are Asian American

Concentration of Hepatitis B Virus in Various Body Fluids

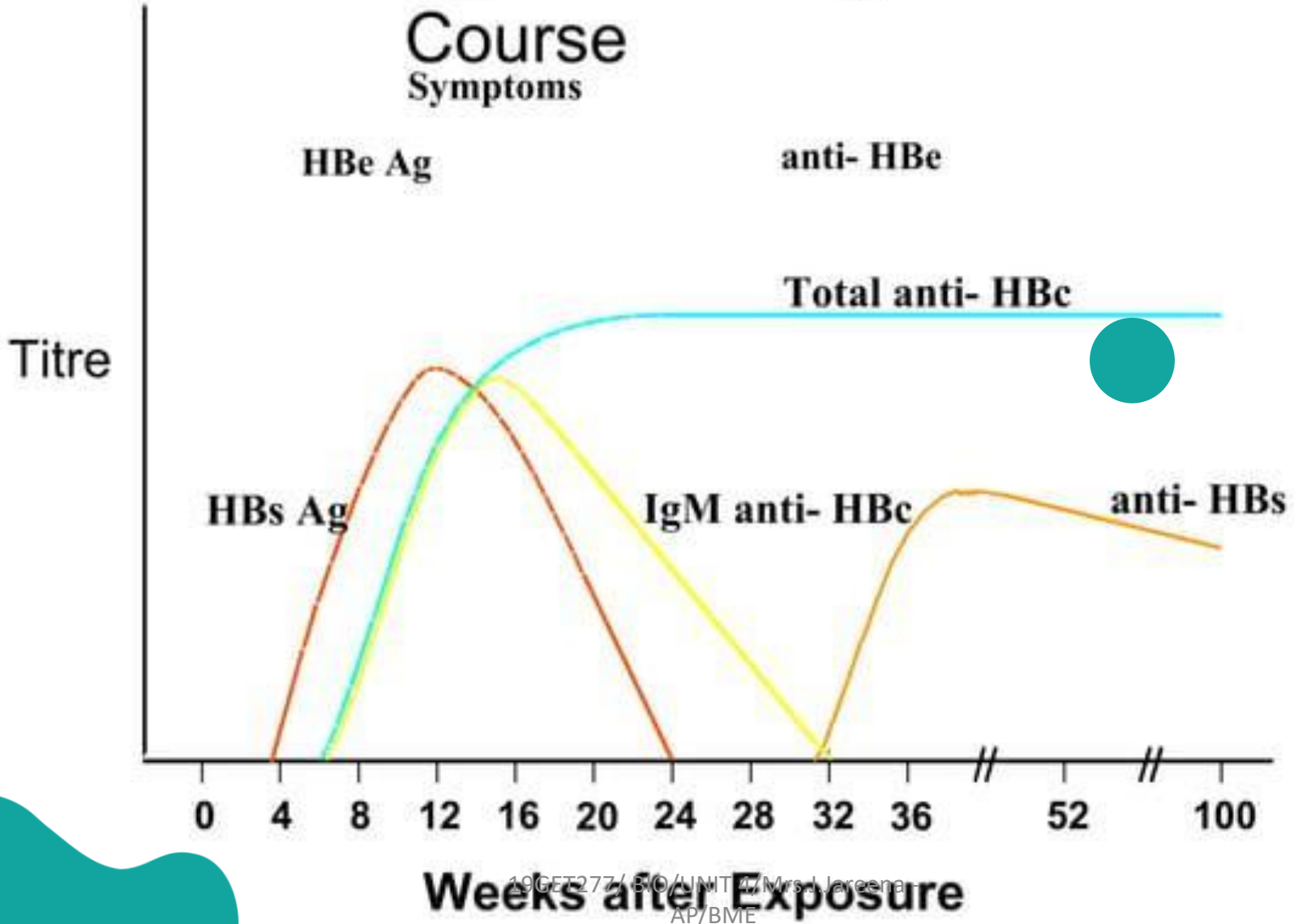
High	Moderate	Low/Not Detectable
blood	semen 精液	[illegible]ine
serum	vaginal fluid 阴道分泌液	feces
wound exudates 伤口渗出液	saliva 唾液	sweat
		tears
		Breast milk

High-risk groups for HBV infection

- People from endemic regions
- Babies of mothers with chronic HBV
- Intravenous drug abusers
- People with multiple sex partners
- Hemophiliacs and other patients requiring blood and blood product treatments
- Health care personnel who have contact with blood
- Residents and staff members of institutions for the mentally retarded

cute Hepatitis B Virus Infection with Recc

Typical Serologic Course



Pathogenesis & Immunity

- Virus enters hepatocytes via blood
- Immune response (cytotoxic T cell) to viral antigens expressed on hepatocyte cell surface responsible for clinical syndrome
- 5 % become chronic carriers (HBsAg > 6 months)
- Higher rate of hepatocellular ca in chronic carriers, especially those who are “e” antigen positive
- Hepatitis B surface antibody likely confers lifelong immunity (IgG anti-HBs)
- Hepatitis B e Ab indicates low transmissibility

6、 Clinical Features

Incubation period: Average 60-90 days
Range 45-180 days

Insidious onset of symptoms.

Tends to cause a more severe disease than Hepatitis A.

Clinical illness (jaundice): <5 yrs, <10%
≥ 5 yrs, 30%-50%
1/3 adults-no symptoms

Clinical Illness at presentation 10 - 15%

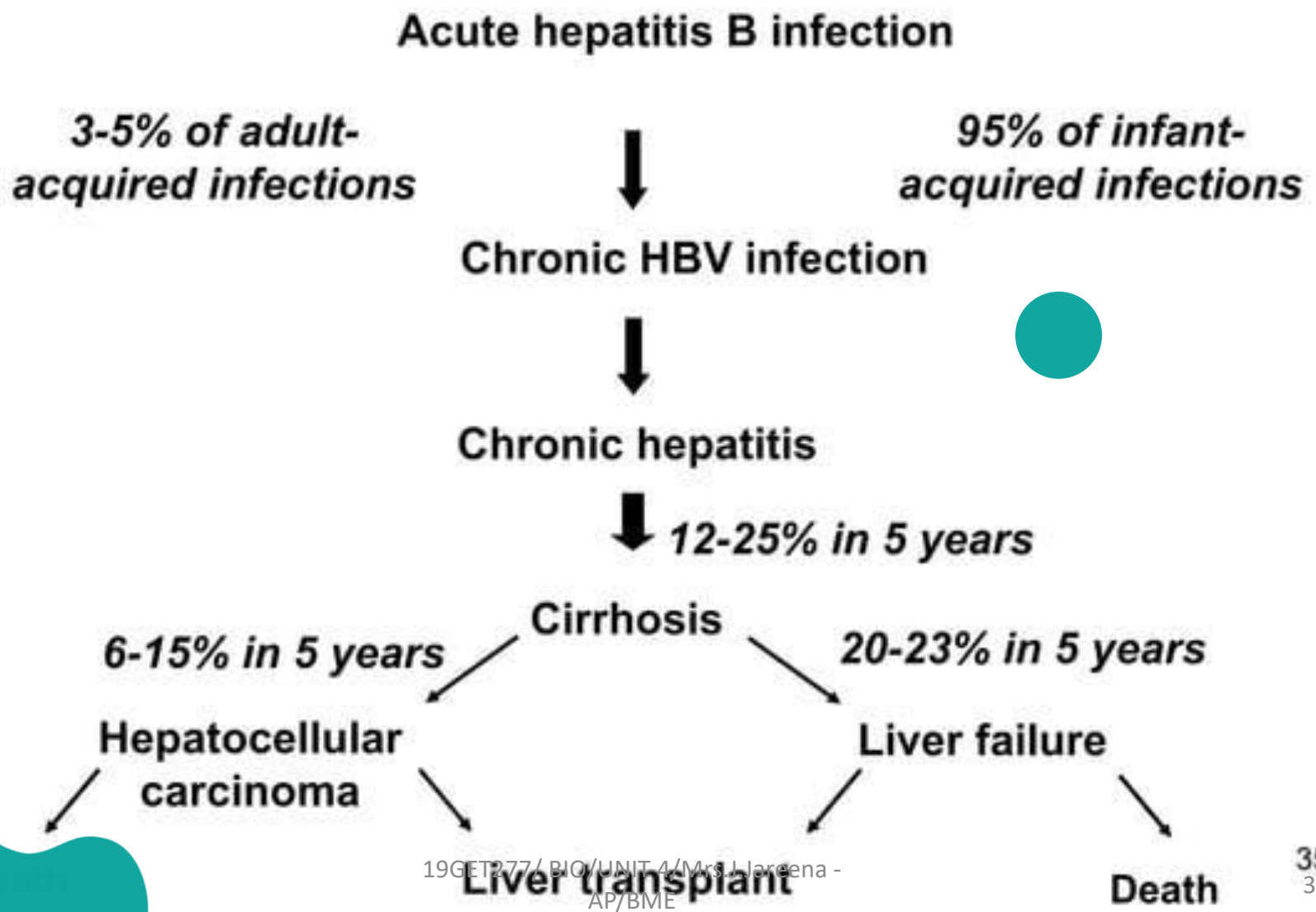
Acute case-fatality rate: 0.5%-1%

Chronic infection: < 5 yrs, 30%-90%
≥ 5 yrs, 2%-10%

More likely in asymptomatic infections

Premature mortality from chronic liver disease: 15%-25%

ssible Outcomes of HBV Infe



7 、 Laboratory Diagnos

Serologic Markers for the Different Phases of Acute and Chronic Hepatitis B Virus Infection

HBsAg	HBeAg	IgM anti-HBc	IgG anti-HBc	Anti-HBs	Anti-HBe	HBV DNA	Interpretation
Acute HBV infection							
+	+	+				+	Early phase
		+				±	Window phase
			+	+	+	-	Recovery phase
Chronic HBV infection							
+	+		+			+	Replicative phase
+			+		+	-	Low, nonreplicative phase
+	±	+				+	Flare-up of chronic HBV
+					+	+	Precore/core promoter mutants

Diagnosis

- A battery of serological tests are used for the diagnosis of acute and chronic hepatitis B infection.
- HBsAg - used as a general marker of infection.
- HBsAb - used to document recovery and/or immunity to HBV infection.
- anti-HBc IgM - marker of acute infection.
- anti-HBcIgG - past or chronic infection.
- HBeAg - indicates active replication of virus and therefore infectiveness.
- Anti-Hbe - virus no longer replicating. However, the patient can still be positive for HBsAg which is made by integrated HBV.
- HBV-DNA - indicates active replication of virus, more accurate than HBeAg especially in cases of escape mutants. Used mainly for monitoring response to therapy.

、 Current Treatment Options

- Interferon alfa (Intron A) （干扰素） Response rate is 30 to 40%.
- Lamivudine (Epivir HBV) （拉米呋啶）
- (relapse ,drug resistance)
- Adefovir dipivoxil (Hepsera)（阿德福韦酯）

Treatment

- Interferon - for HBeAg +ve carriers with chronic active hepatitis. Response rate is 30 to 40%.
  - alpha-interferon 2b (original)
  - alpha-interferon 2a (newer, claims to be more efficacious and efficient)
- Lamivudine - a nucleoside analogue reverse transcriptase inhibitor. Well tolerated, most patients will respond favorably. However, tendency to relapse on cessation of treatment. Another problem is the rapid emergence of drug resistance.
- Adefovir – less likely to develop resistance than Lamivudine and may be used to treat Lamivudine resistance HBV. However more expensive and toxic
- Entecavir – most powerful antiviral known, similar to Adefovir
- Successful response to treatment will result in the disappearance of HBsAg, HBV-DNA, and seroconversion to HBeAg.

Prevention

- **Vaccination**

  - highly effective recombinant vaccines

- **Hepatitis B Immunoglobulin** (HBIG)

  -exposed within 48 hours of the incident/ neonates whose mothers are HBsAg and HBeAg positive.

- **Other measures**

  -screening of blood donors, blood and body fluid precautions.

Prevention

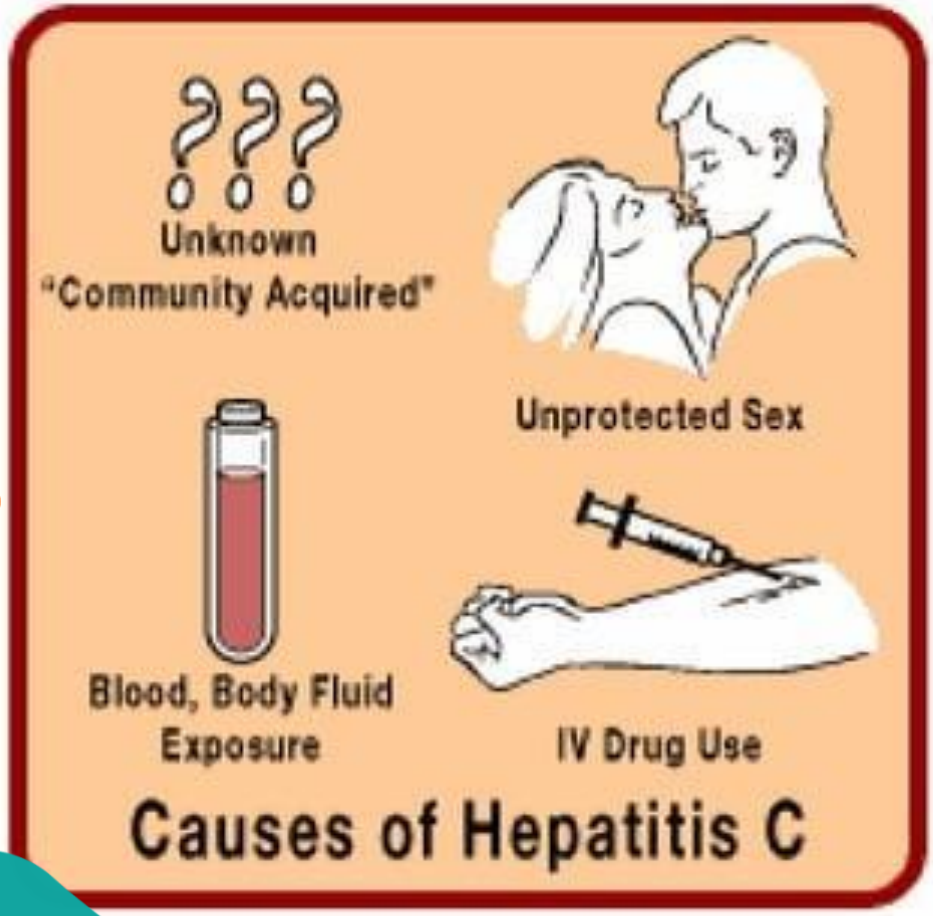
- Vaccination - highly effective recombinant vaccines are now available. Vaccine can be given to those who are at increased risk of HBV infection such as health care workers. It is also given routinely to neonates as universal vaccination in many countries.
- Hepatitis B Immunoglobulin - HBIG may be used to protect persons who are exposed to hepatitis B. It is particular efficacious within 48 hours of the incident. It may also be given to neonates who are at increased risk of contracting hepatitis B i.e. whose mothers are HBsAg and HBeAg positive.
- Other measures - screening of blood donors, blood and body fluid precautions.

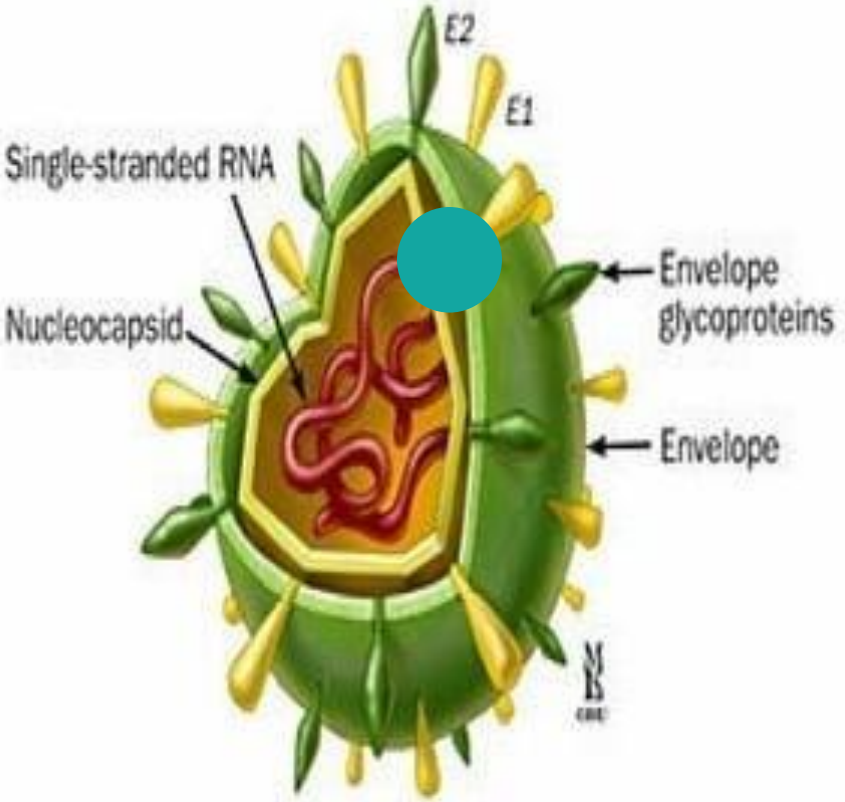
Hepatitis B Vaccine

- Infants: several options that depend on status of the mother
  - If mother HBsAg negative: birth, 1-2m,6-18m
  - If mother HBsAg positive: vaccine and Hep B immune globulin within 12 hours of birth, 1-2m, <6
- Adults

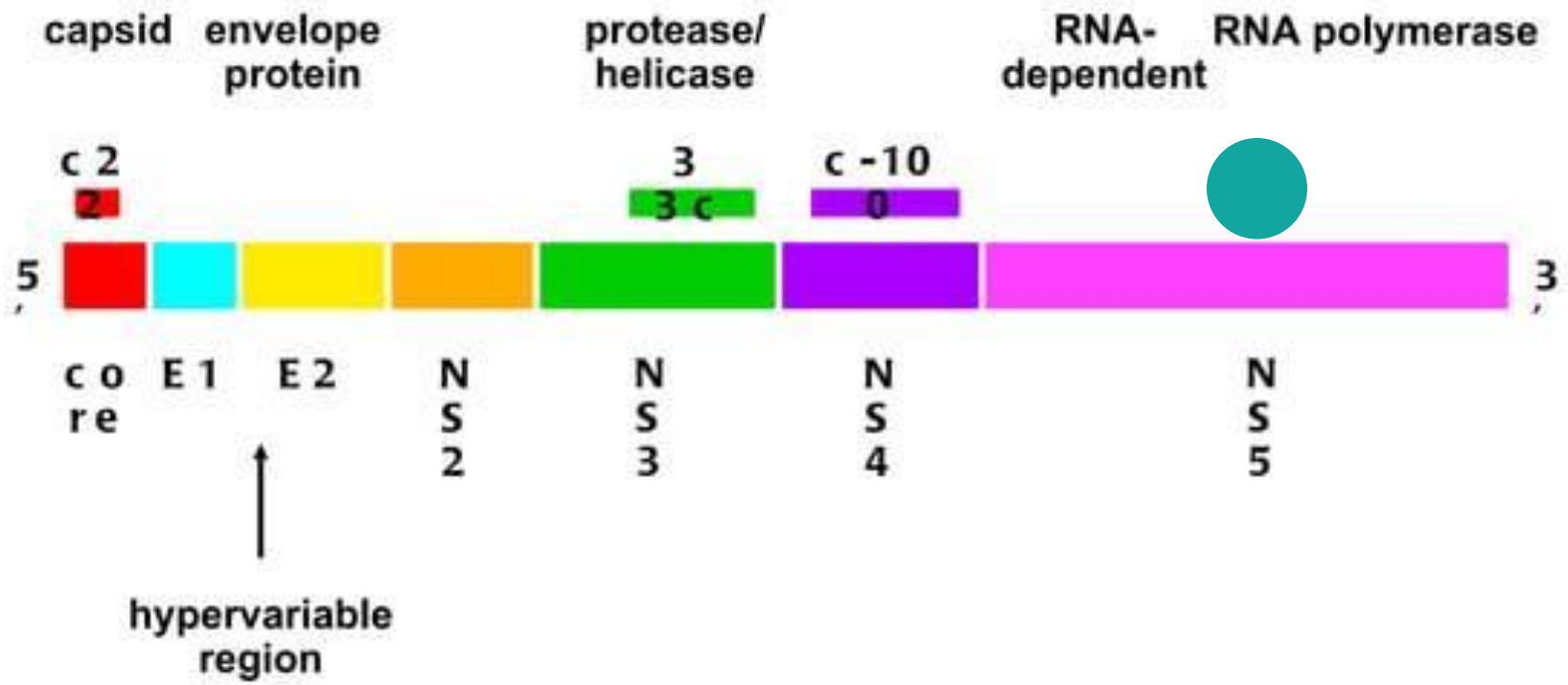
  * 0,1, 6 months
- Vaccine recommended in
  - All those aged 0-18
  - Those at high risk

HEPATITIS C VIRION: spherical, icosahedral, NUCLEIC ACID: ss (+) RNA





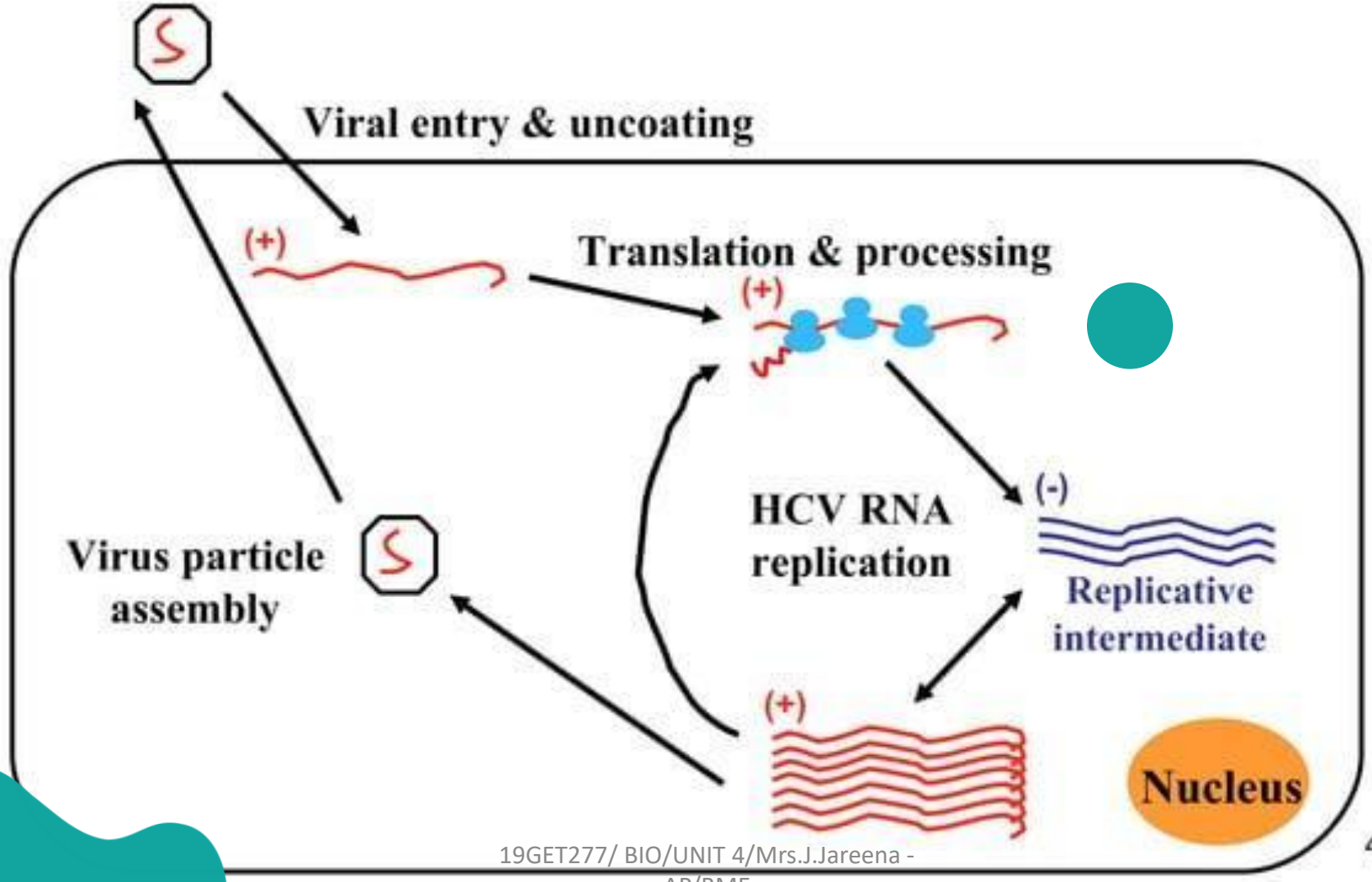
Hepatitis C Virus



Hepatitis C Virus

- Genome resembled that of a flavivirus positive stranded RNA genome of around 10,000 bases
- 1 single reading frame, structural genes at the 5' end, the non-structural genes at the 3' end.
  enveloped virus, virion thought to 30-60nm in diameter
- morphological structure remains unknown
- HCV has been classified into a total of six genotypes (type 1 to 6) on the basis of phylogenetic analysis
- Genotype 1 and 4 has a poorer prognosis and response to interferon therapy
- In Hong Kong, genotype 1 accounts for around 67% of cases and genotype 6 around 25%.

HCV replicates exclusively in the cytoplasm via an RNA intermediate



Hepatitis C - Clinical Features

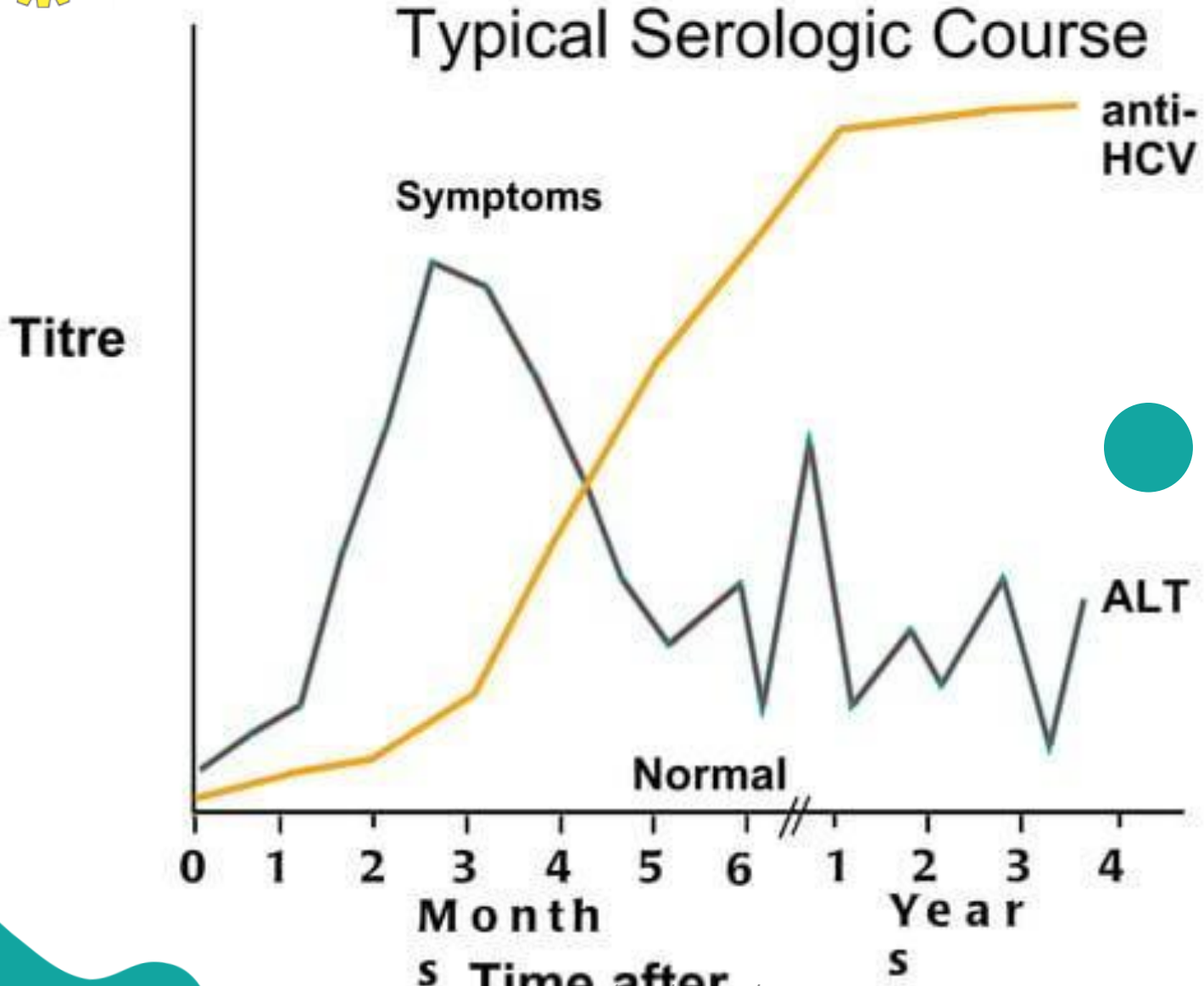
Incubation period:	Average 6-7 wks Range 2-26 wks
Clinical illness (jaundice): (20-30%)	30-40%
Chronic hepatitis:	70%
Persistent infection:	85-100%
Immunity:	No protective antibody response identified

Chronic Hepatitis C Infection

- The spectrum of chronic hepatitis C infection is essentially the same as chronic hepatitis B infection.

- All the manifestations of chronic hepatitis B infection may be seen, albeit with a lower frequency i.e. chronic persistent hepatitis, chronic active hepatitis, cirrhosis, and hepatocellular carcinoma.

Hepatitis C Virus Infection



Risk Factors Associated with Transmission of HCV

- Transfusion or transplant from infected donor
- Injecting drug use
- Hemodialysis (yrs on treatment)
- Accidental injuries with needles/sharps
- Sexual/household exposure to anti-HCV-positive contact
- Multiple sex partners
- Birth to HCV-infected mother

Laboratory Diagnosis

- HCV antibody - generally used to diagnose hepatitis C infection. Not useful in the acute phase as it takes at least 4 weeks after infection before antibody appears.

- HCV-RNA - various techniques are available e.g. PCR and branched DNA. May be used to diagnose HCV infection in the acute phase. However, its main use is in monitoring the response to antiviral therapy.

- HCV-antigen - an EIA for HCV antigen is available. It is used in the same capacity as HCV-RNA tests but is much easier to carry out.

HCV RNA (PCR testing)

H Virus load

V Lower detection limit can be 10-615 IU/ml

L NOT a predictor of disease severity: a high viral load does not mean the liver disease is more severe, and a low viral load does not mean the patient is ok and does not need therapy!

p Helps predict response rate to treatment (lower means a higher chance of cure with therapy)

d to monitor response during treatment

Prognostic Tests

- Genotyping – genotype 1 and 4 have a worse prognosis overall and respond poorly to interferon therapy. A number of commercial and in-house assays are available.
  - Genotypic methods – DNA sequencing, PCR-hybridization e.g. INNO-LIPA.
  - Serotyping – particularly useful when the patient does not have detectable RNA.

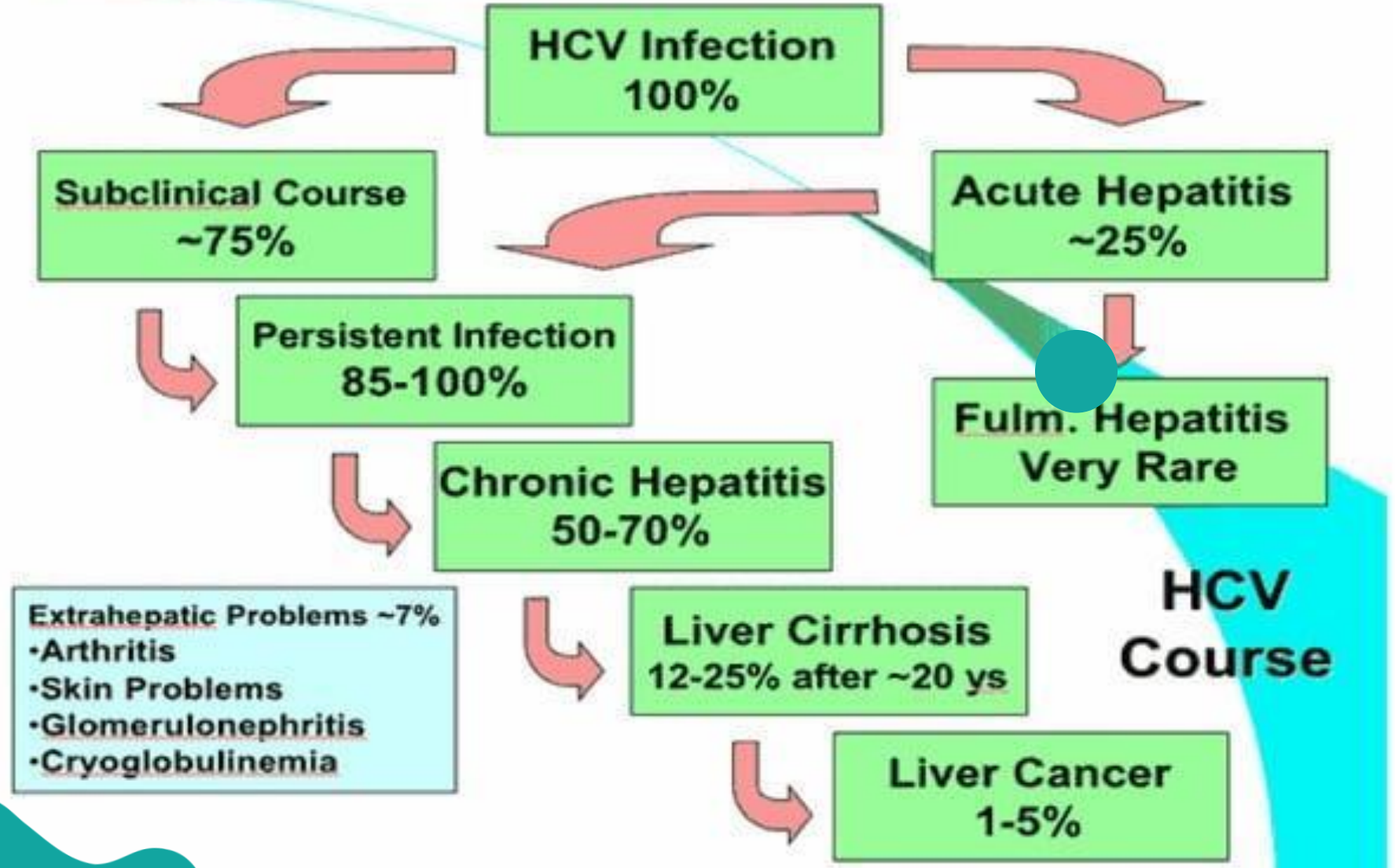
- Viral Load – patients with high viral load are thought to have a poorer prognosis. Viral load is also used for monitoring response to IFN therapy. A number of commercial and in-house tests are available.

Treatment

- Interferon - may be considered for patients with chronic active hepatitis. The response rate is around 50% but 50% of responders will relapse upon withdrawal of treatment.

- Ribavirin - there is less experience with ribavirin than interferon. However, recent studies suggest that a combination of interferon and ribavirin is more effective than interferon alone.

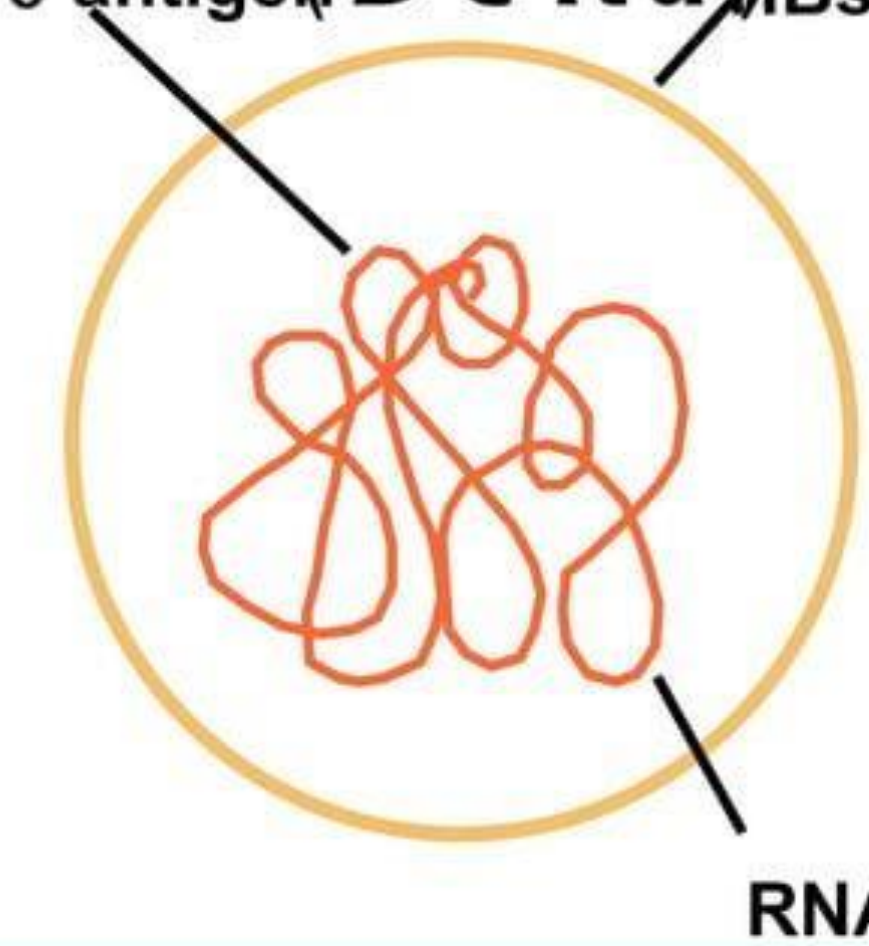
OUTCOMES of HCV hepatitis

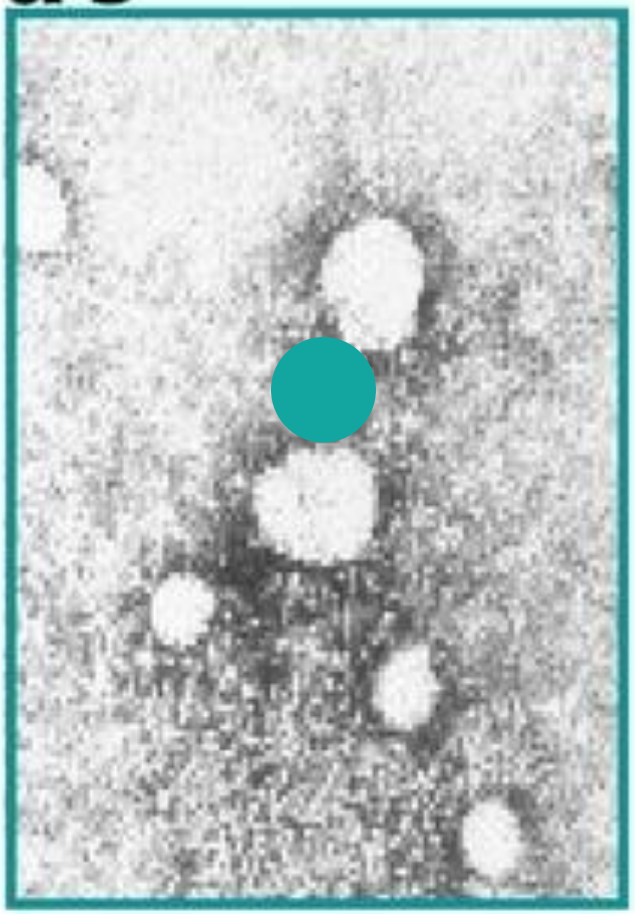


Prevention of Hepatitis C

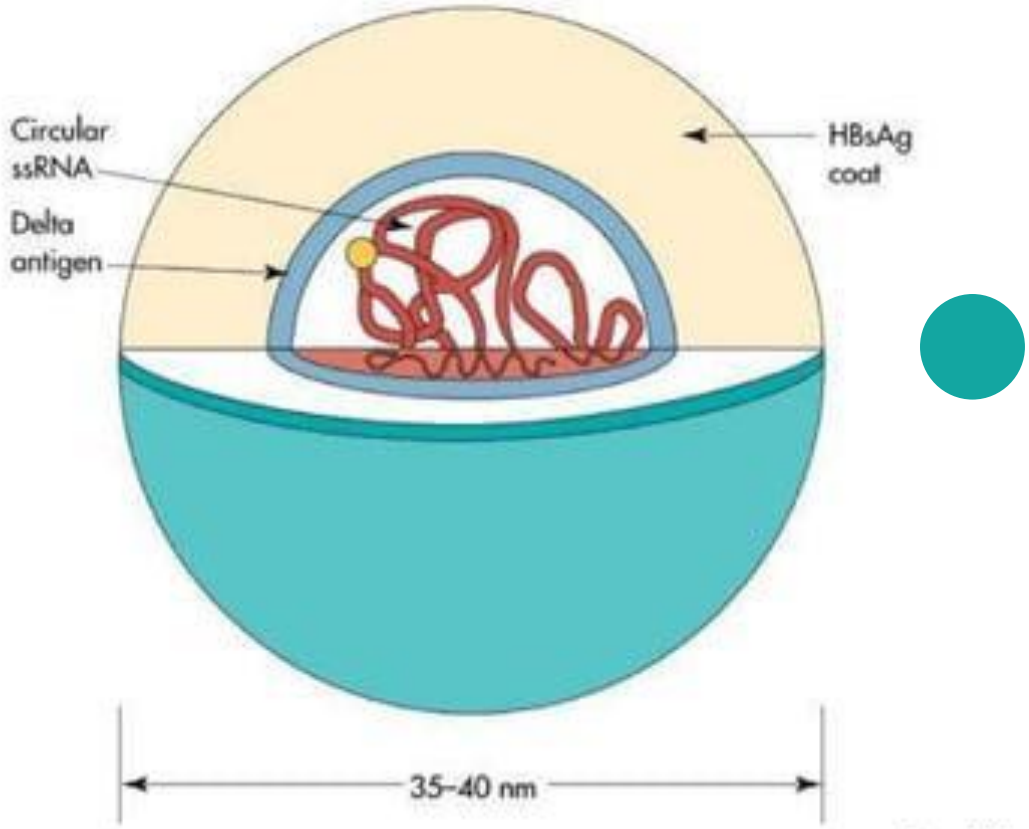
- Screening of blood, organ, tissue donors
- High-risk behavior modification
- Blood and body fluid precautions

Hepatitis D (Delta) Virus





Hepatitis Delta Virion



From Murray et. al., Medical Microbiology 5th edition, 2005, Chapter 66, published by Mosby Philadelphia,.

Figure 66-14

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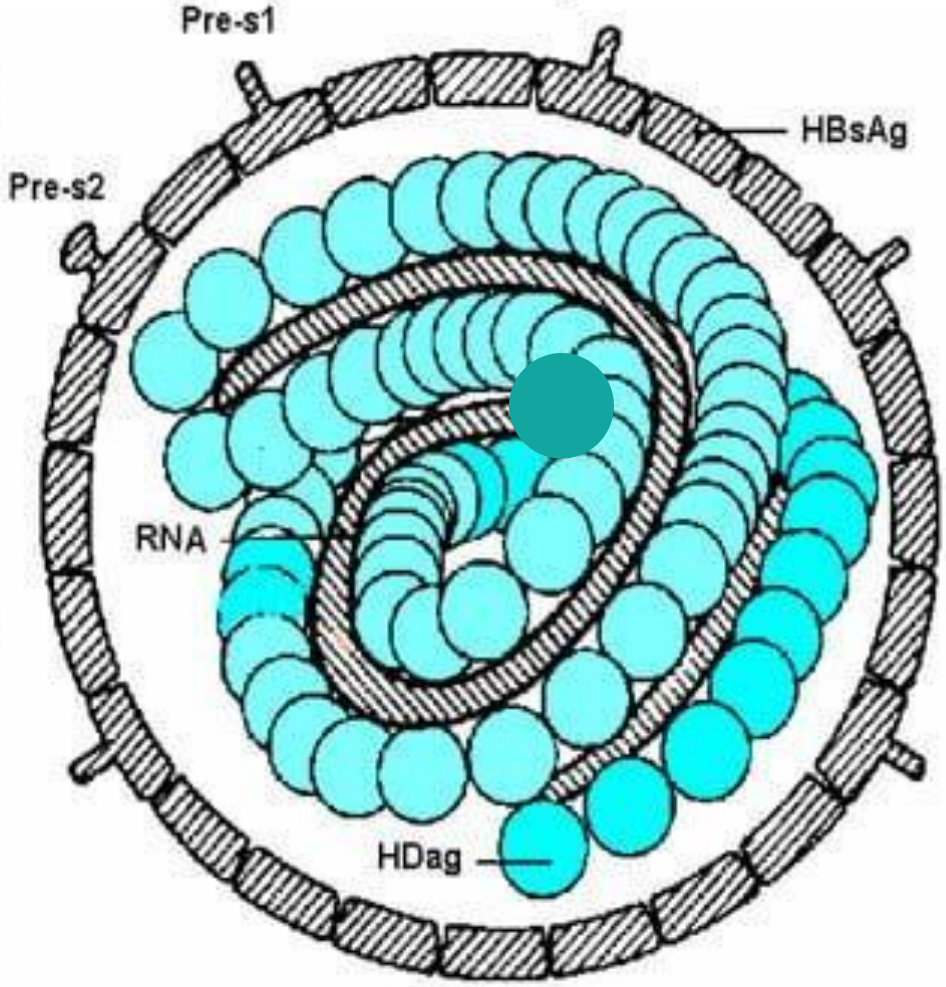
HEPATITIS D VIRUS (HDV, DELTA AGENT)

VIRION: spherical, 36-38 nm,
HBV capsid, HDV nucleoprotein
NUCLEIC ACID: (-) ss RNA, circular
Satellite virus : replicates only
in the presence of HBV



Hepatitis D Virus

- The delta agent is a defective virus which shows similarities with the viroids in plants.
- The agent consists of a particle 35 nm in diameter consisting of the delta antigen surrounded by an outer coat of HBsAg.
- The genome of the virus is very small and consists of a single-stranded RNA

The HDV genome

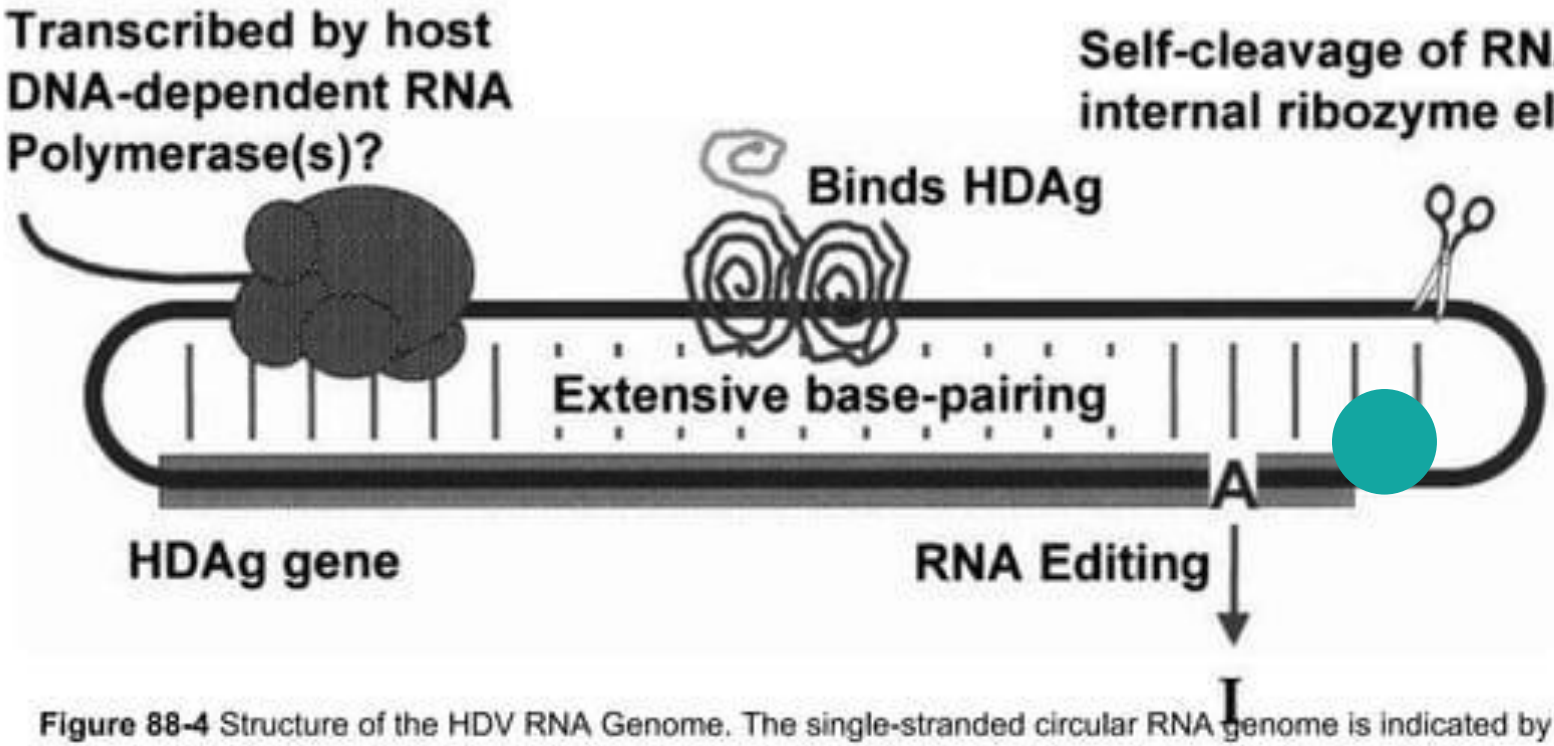


Figure 88-4 Structure of the HDV RNA Genome. The single-stranded circular RNA genome is indicated by the *heavy black continuous line*. The genome has the ability to form an unbranched rod structure, in which approximately 70% of the bases are engaged in Watson-Crick pairs with counterparts from the opposite side of the circular RNA. In this unbranched rod structure, the region encoding HDAg (nt 1598-957) is on one side. The RNA editing site is at position 1012 in the antigenome. The region on the right-hand side contains the autocatalytic cleavage sites (ribozymes), one in the genome (nt 686) and the other in the antigenome (nt 900). The genome binds HDAg and is transcribed by a host DNA–dependent RNA polymerase.

Fields Virology 4th edition, 2002, Chapter 88, Lippincott, Williams and Wilkins, 2002 Fig. 88-4

Hepatitis D - Clinical Features

- Coinfection

  severe acute disease.

  low risk of chronic infection.

- Superinfection

  usually develop chronic HDV infection.

  high risk of severe chronic liver disease.

  may present as an acute hepatitis.

Consequences of hepatitis B and delta virus infection

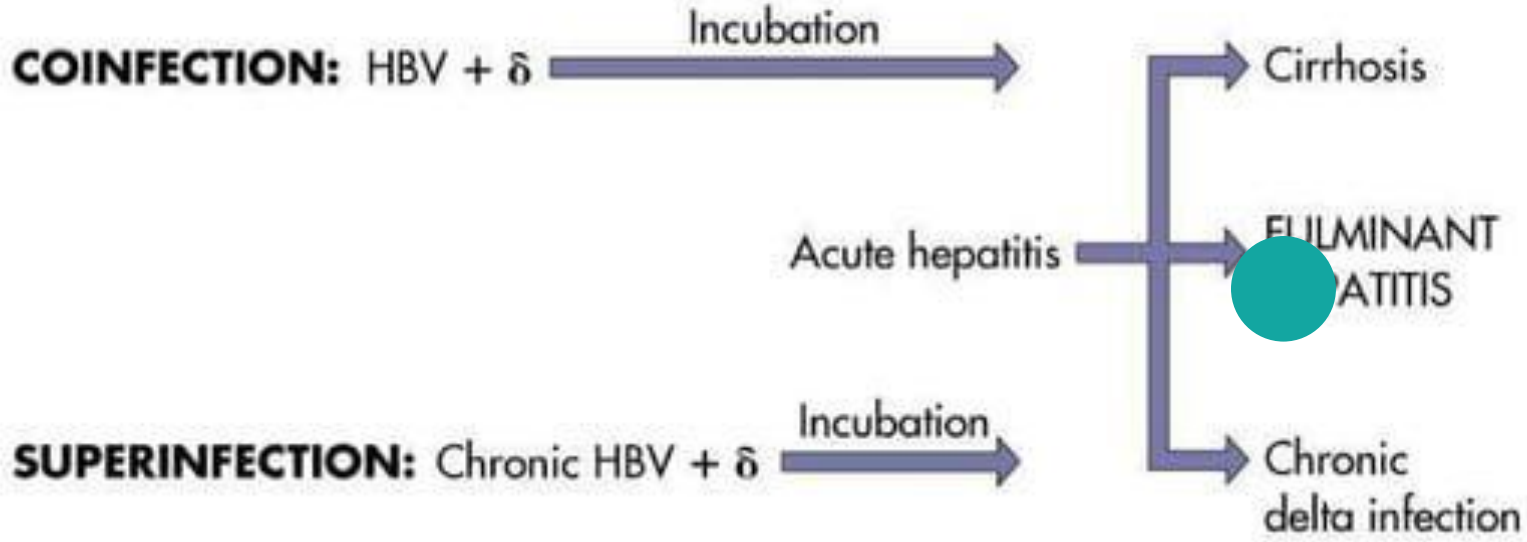


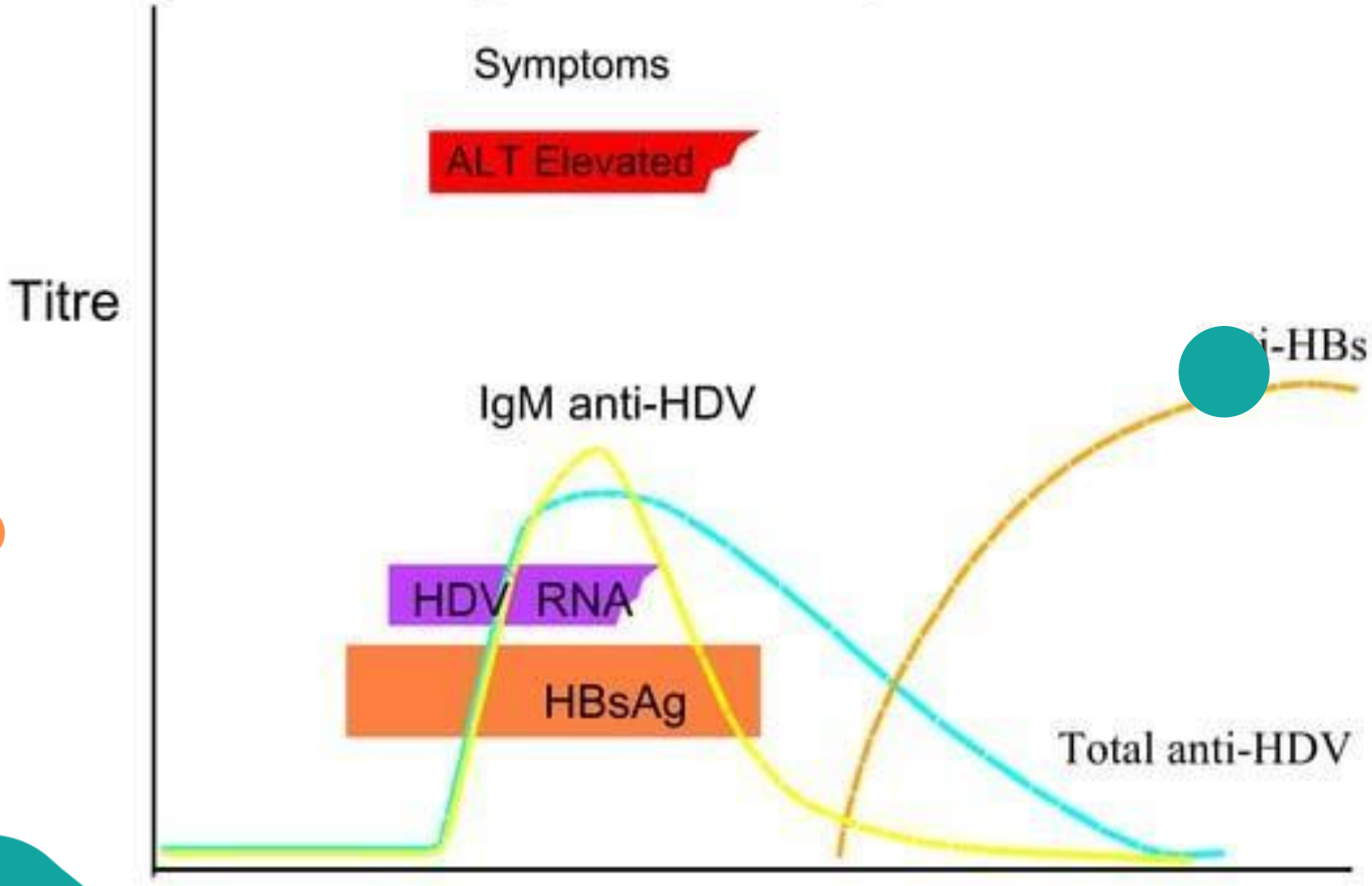
Figure 66-15. Consequences of deltavirus infection. Deltavirus (d) requires the presence of hepatitis B virus (HBV) infection. Superinfection of a person already infected with HBV (carrier) causes more rapid, severe progression than co-infection (*shorter arrow*).

Hepatitis D Virus Modes of Transmission

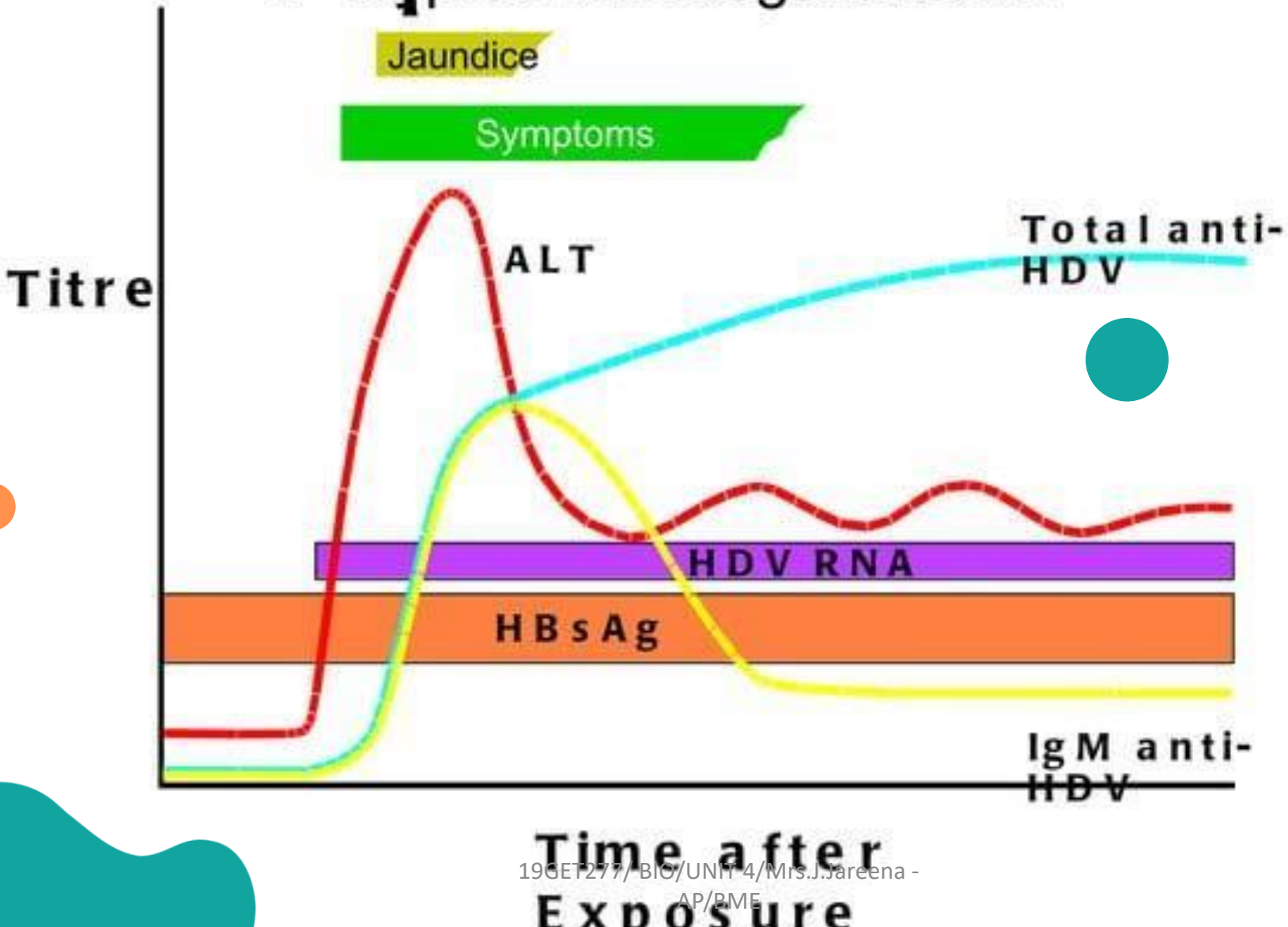
- Percutaneous exposures
  - injecting drug use
- Permucosal exposures
  - sex contact

HBV - HDV Coinfection

Typical Serologic Course



HBV – HDV Superinfection



Hepatitis D - Preventions

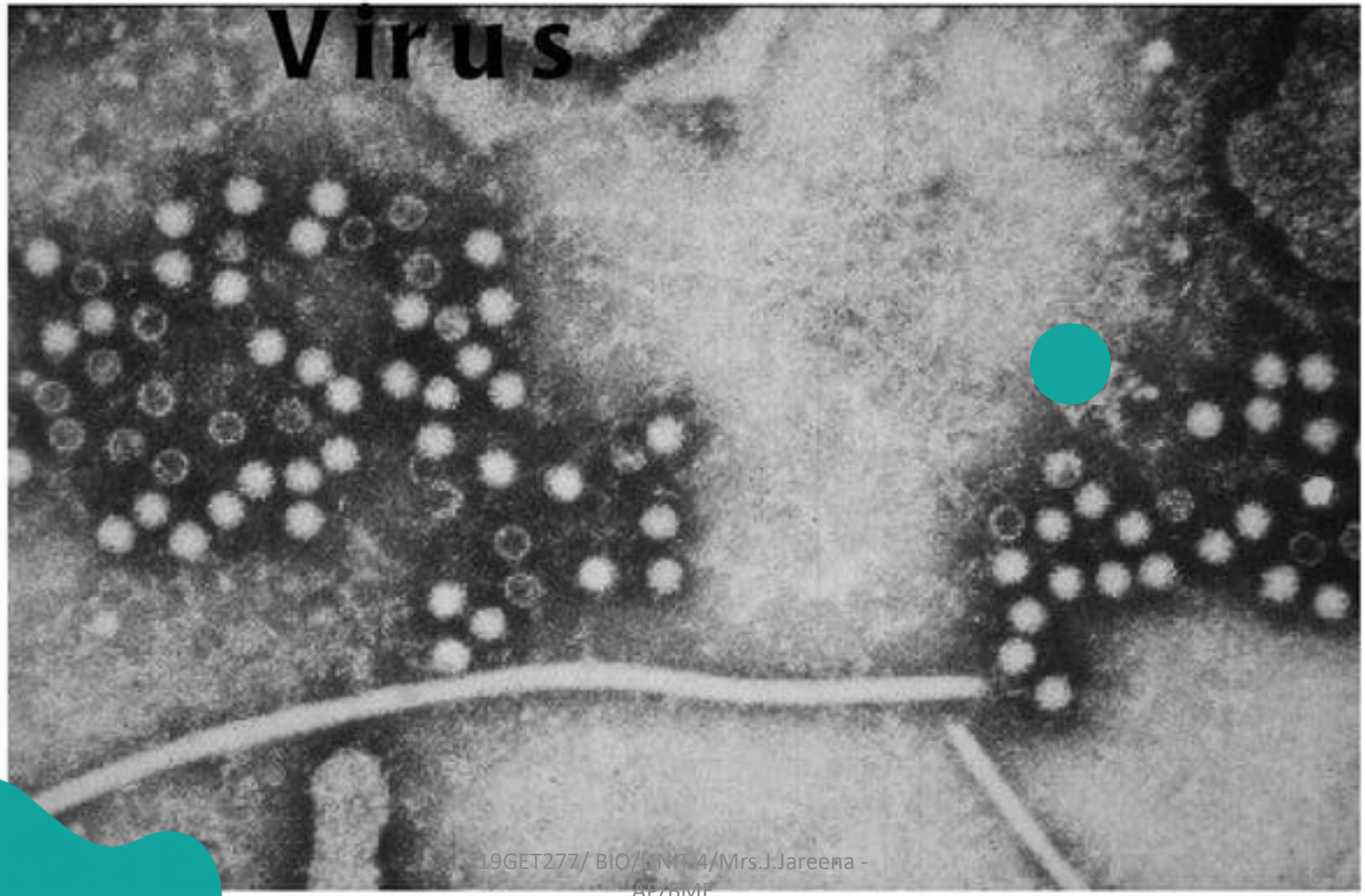
- HBV-HDV Coinfection

  Pre or post exposure prophylaxis to prevent HBV infection.

- HBV-HDV Superinfection

  Education to reduce risk behaviors among persons with chronic HBV infection.

Hepatitis E Virus



Hepatitis E Virus

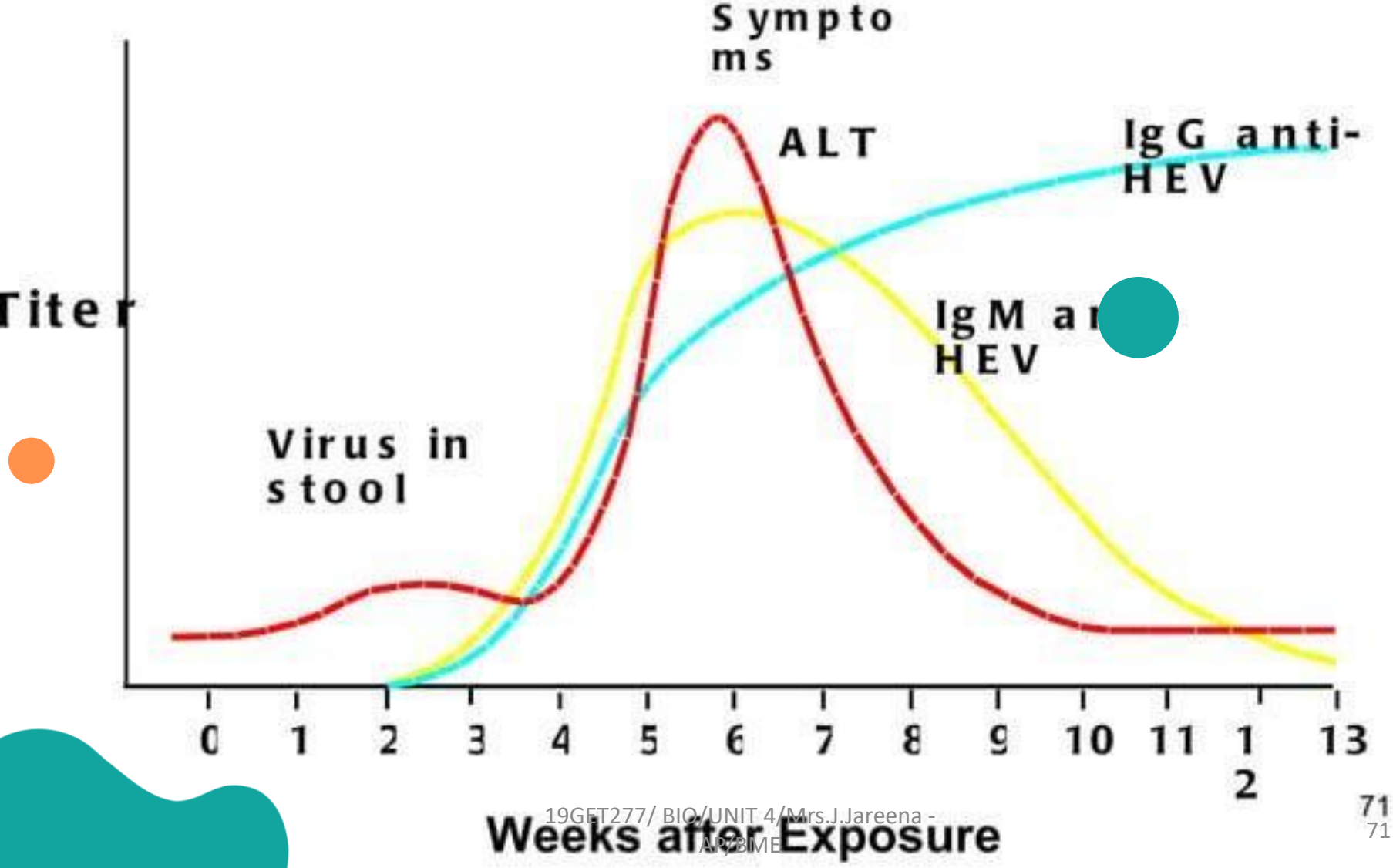
- Calicivirus-like viruses
- unenveloped RNA virus, 32-34nm in diameter
- +ve stranded RNA genome, 7.6 kb in size.
- very labile and sensitive
- only be cultured recently

Hepatitis E - Clinical Features

- Incubation period: Average 40 days
  Range 15-60 days
- Case-fatality rate: Overall, 1%-3%
  Pregnant women, 15%-25%
- Illness severity: Increased with age
- Chronic sequelae: None identified

Hepatitis E Virus Infection

Typical Serologic Course



Hepatitis E - Epidemiologic Features

- Most outbreaks associated with faecally contaminated drinking water.
- Several other large epidemics have occurred since the Indian subcontinent and the USSR, China, Africa and Mexico.
- In the United States and other nonendemic areas, where outbreaks of hepatitis E have not been documented to occur, a low prevalence of anti-HEV (<2%) has been found in healthy populations. The source of infection for these persons is unknown.
- ...mal person-to-person transmission.

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Prevention and Control Measures for Travelers to HEV-Endemic Regions

- Avoid drinking water (and beverages with ice) of unknown purity, uncooked shellfish, and uncooked fruit/vegetables not peeled or prepared by traveler.
- IG prepared from donors in Western countries does not prevent infection.
- Unknown efficacy of IG prepared from donors in endemic areas.
- Vaccine?

HEPATITIS G VÍRUS

FLAVIRUS: similar morphology and genome

◊ in risk groups: acute, chronic and fulminant hepatitis

◊ transmission: blood (mother- newborn babies)

◊ prevalence is higher in HCV infected people