Laboratory diagnosis of HBV serologic marker

Pei He

hepei839872218@126.com

Introduction



He Pei, attending physician, Doctor of Medicine, and master's supervisor. Head of the Immunology Group in the Medical Laboratory Department at Yan'an Hospital of Kunming. Selected as a trainee for the seventh batch of academic and technical leaders at Yan'an Hospital of Kunming. Member of the Kunming Society of Microbiology and Immunology, and member of the Yunnan Provincial Committee of Combined Traditional Chinese and Western Laboratory Medicine. Review expert for medical laboratory new media and the Chinese Clinical Laboratory Management e-journal.

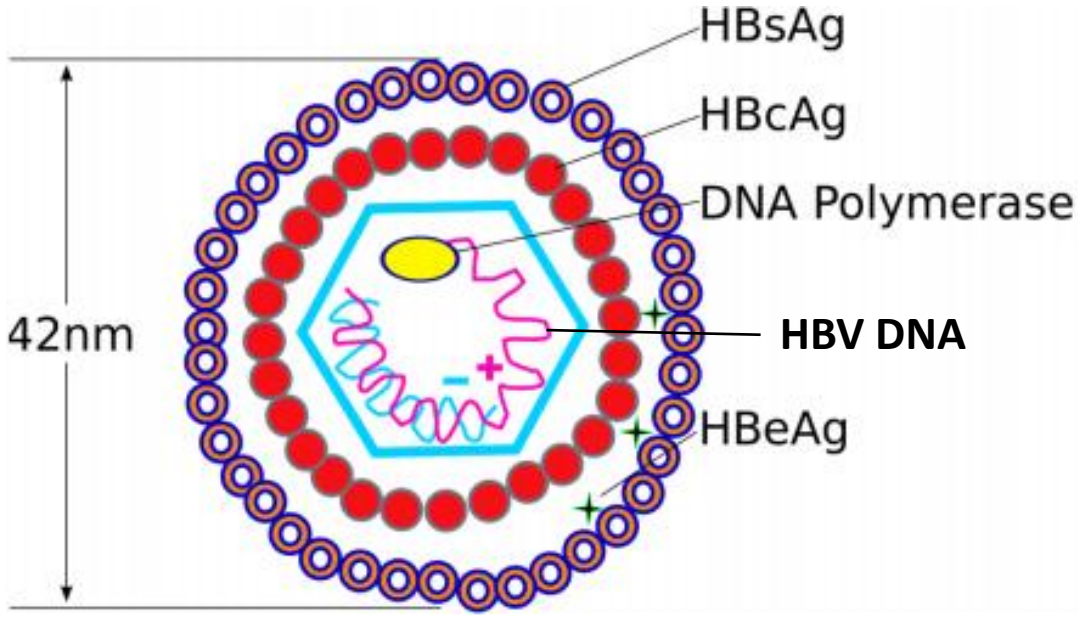
Learning objectives

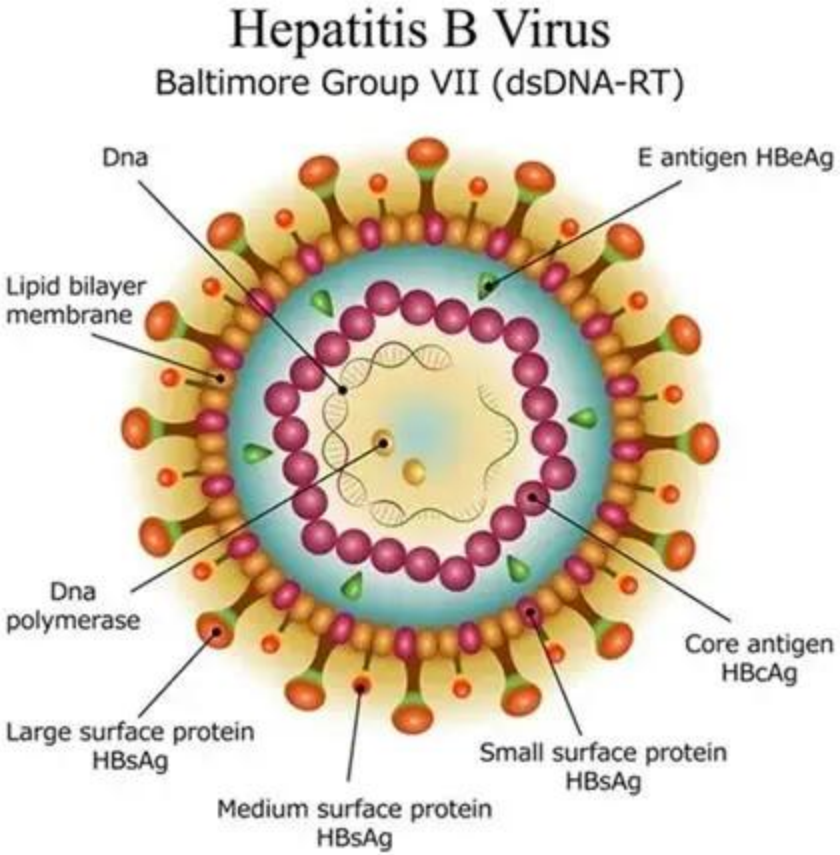
① Master various serological markers of HBV infection

② Understood the testing approach in HBV

③ Know about Clinical Significance of HBV serologic marker

Structure of HBV viral particle



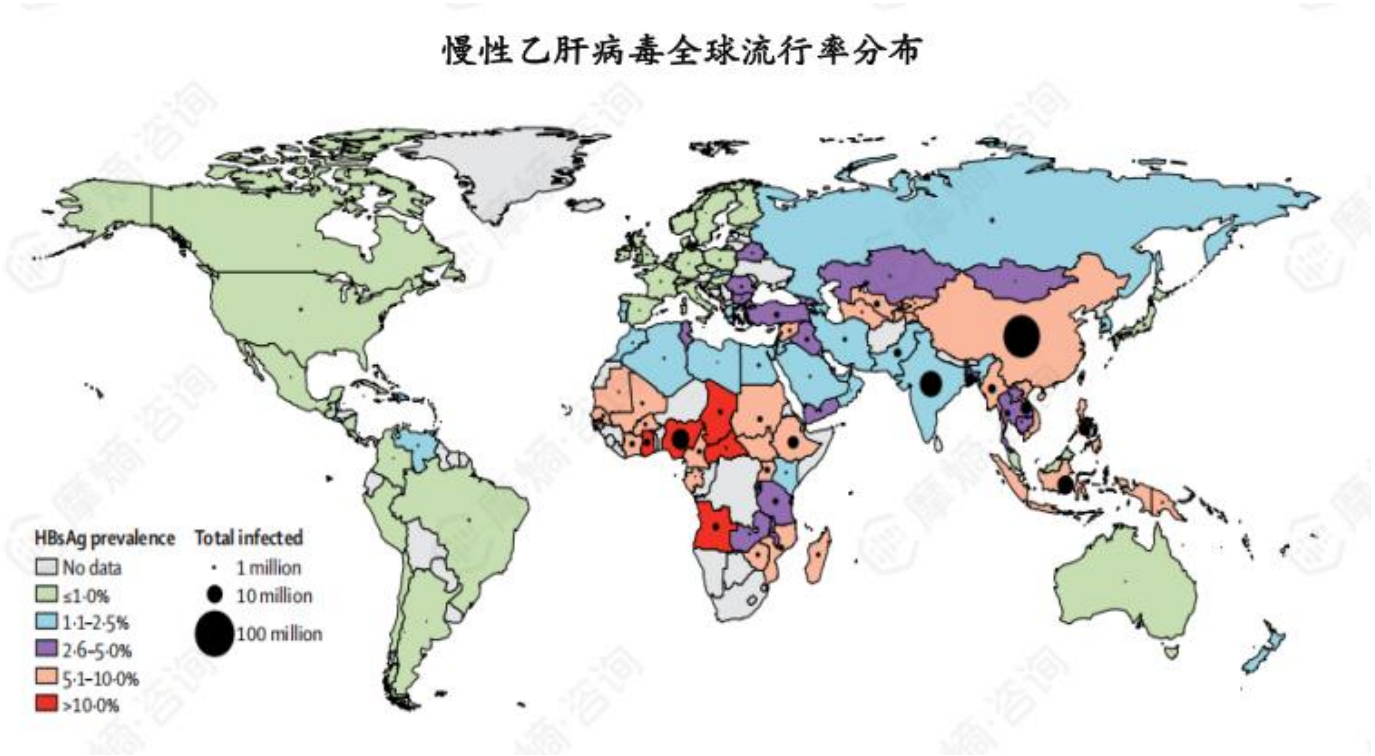


The hepatitis B virus has two parts: an outer shell and a core.

two different mRNAs: a 2.4 kb mRNA translates **LHBs,** and a 2.1 kb mRNA translates **MHBs and SHBs.**

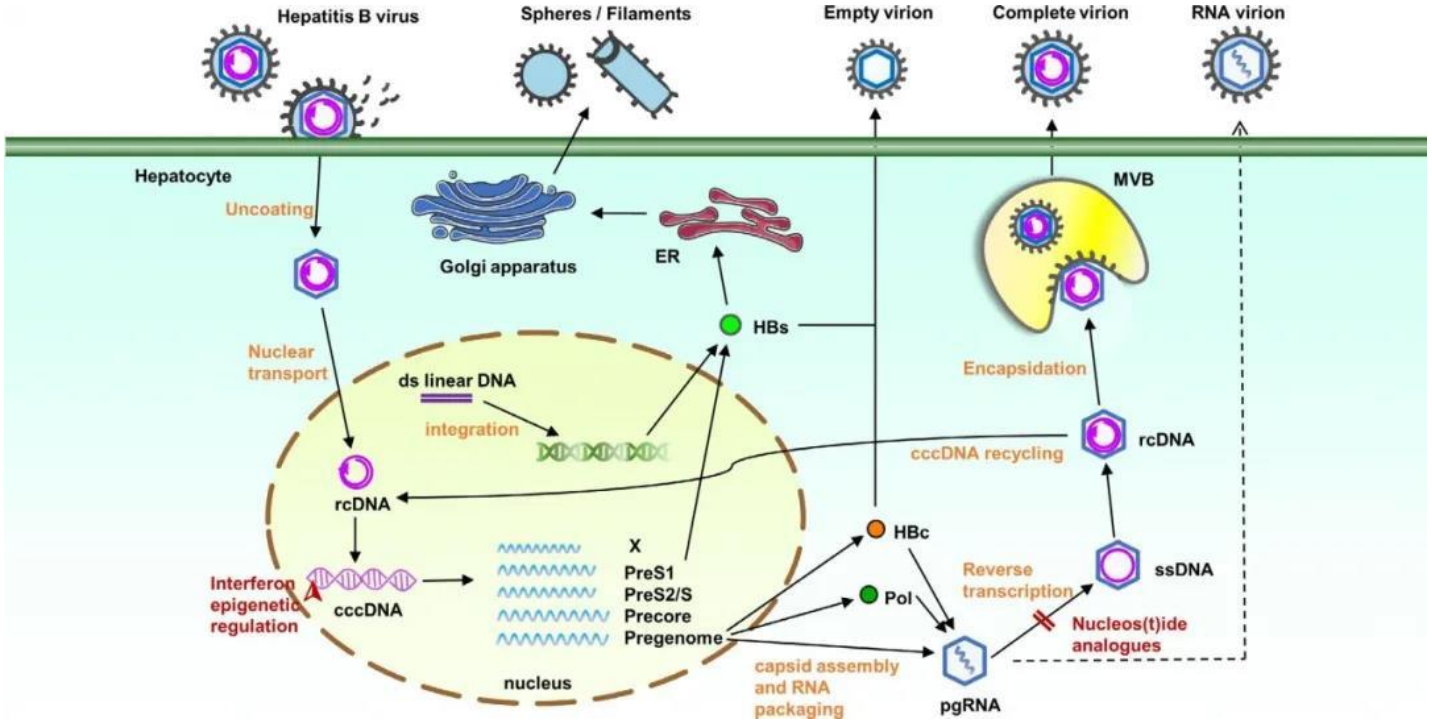
Epidemiology

Hepatitis B virus (HBV) is a **partially circular double-stranded DNA virus** and a major pathogenic factor of viral hepatitis, posing a serious threat to human health.According to research data published in The Lancet, there are significant differences in the prevalence and infection rates of hepatitis B across different regions. Globally, the prevalence of hepatitis B is higher in Asia and Africa, with countries such as **China, India, and Nigeria** being highly endemic for hepatitis B.

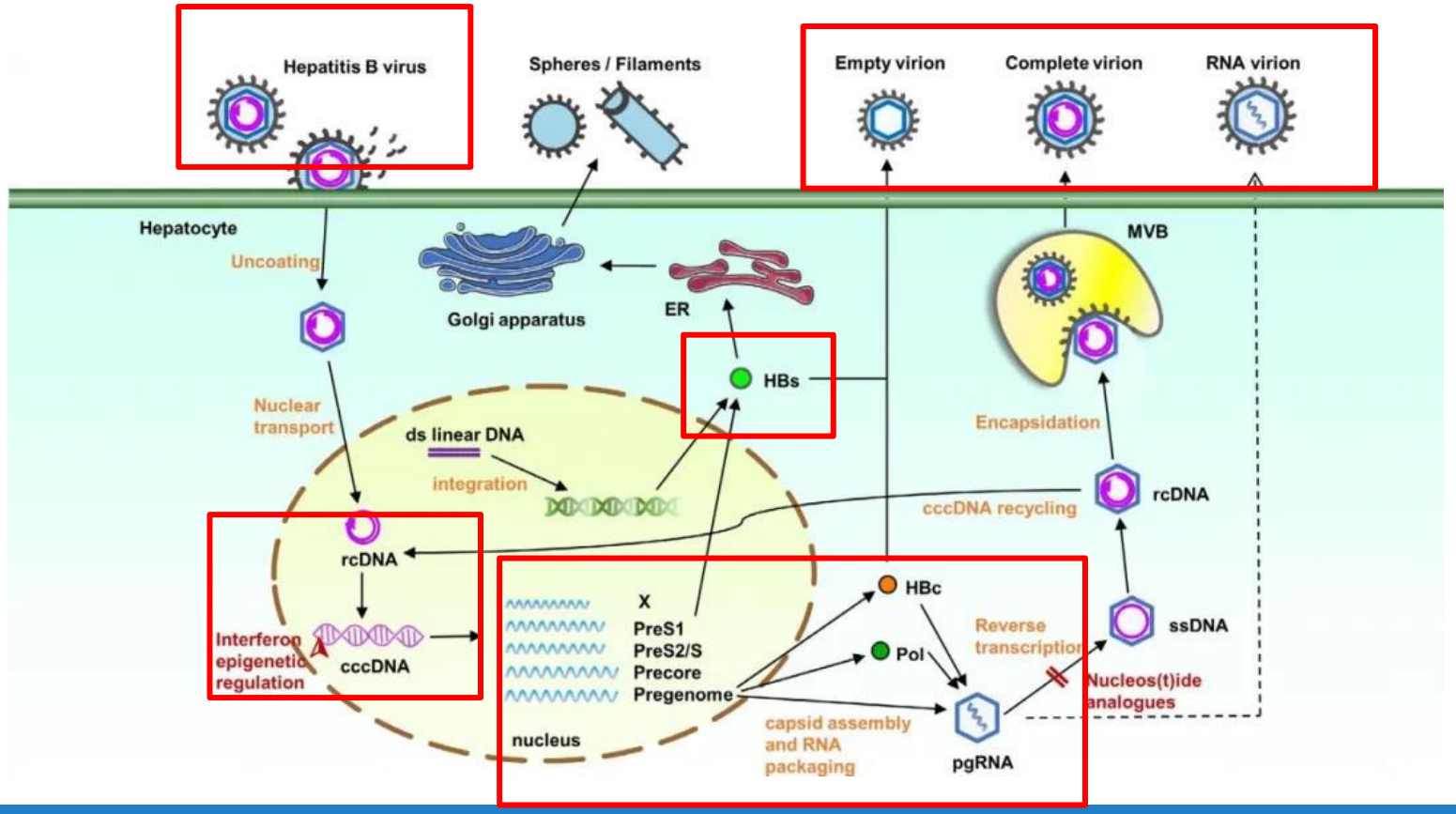


The early steps of HBV entry Upon HBV entry into the host, the Dane particles, which are one of the particulate forms of **HBsAg** in the blood of patients infected with hepatitis B virus (HBV), are concentrated on the low-affinity glycosaminoglycans (GAGS) on the cell surface. The PreS1 domain of the HBV large envelope protein (LHBs) and its receptor sodium taurocholate cotransport polypeptide (**NTCP) share a high afnity**, and its auxiliary receptor epidermal growth factor receptor (EGFR) is bound . This internalization complex is linked to N-glycosylated **NTCP with E-cadherin**. This combination allows NTCP to relocate to the plasma membrane.

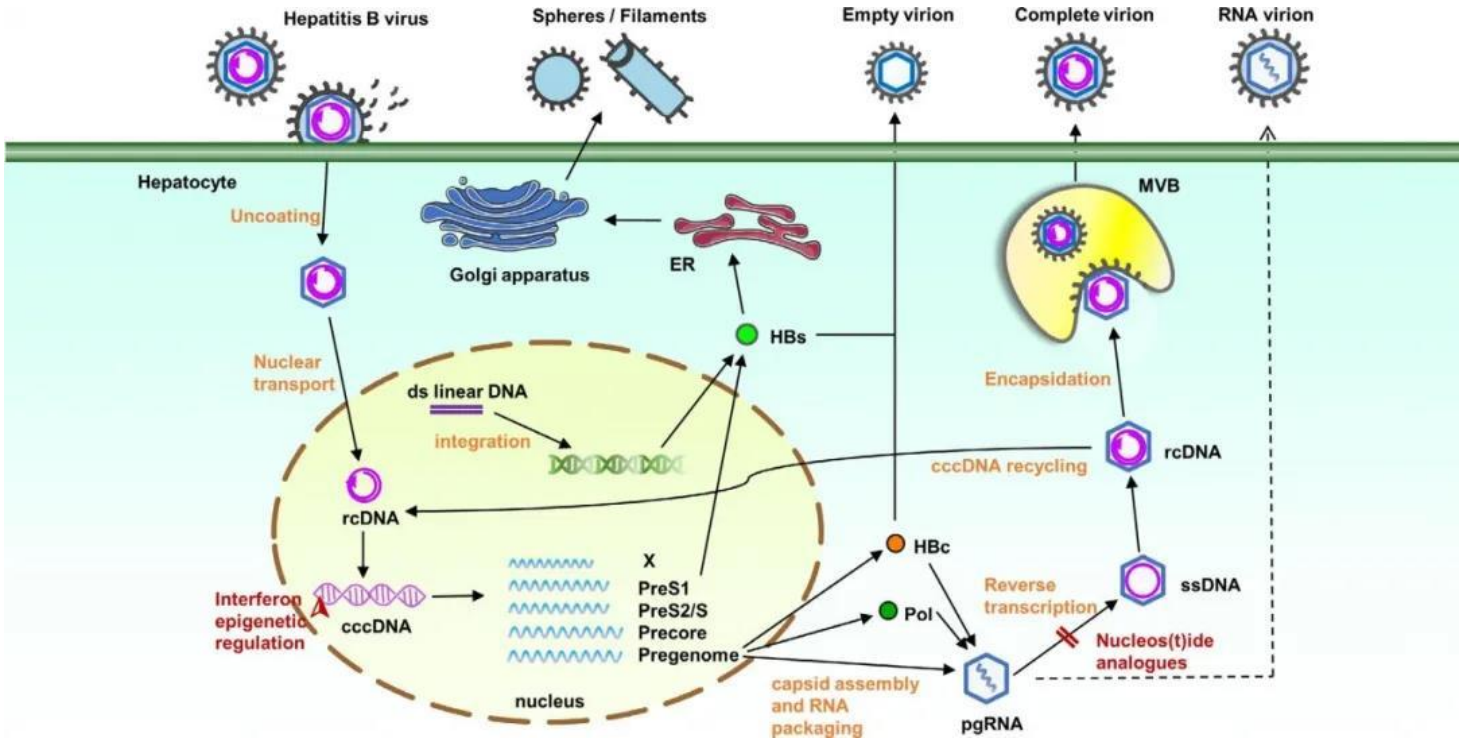
Moreover, a new study revealed that NTCP is needed for de novo infection . Whether the coreceptors and host cytokines are required for hepatitis B to enter liver cells has not been fully elucidated

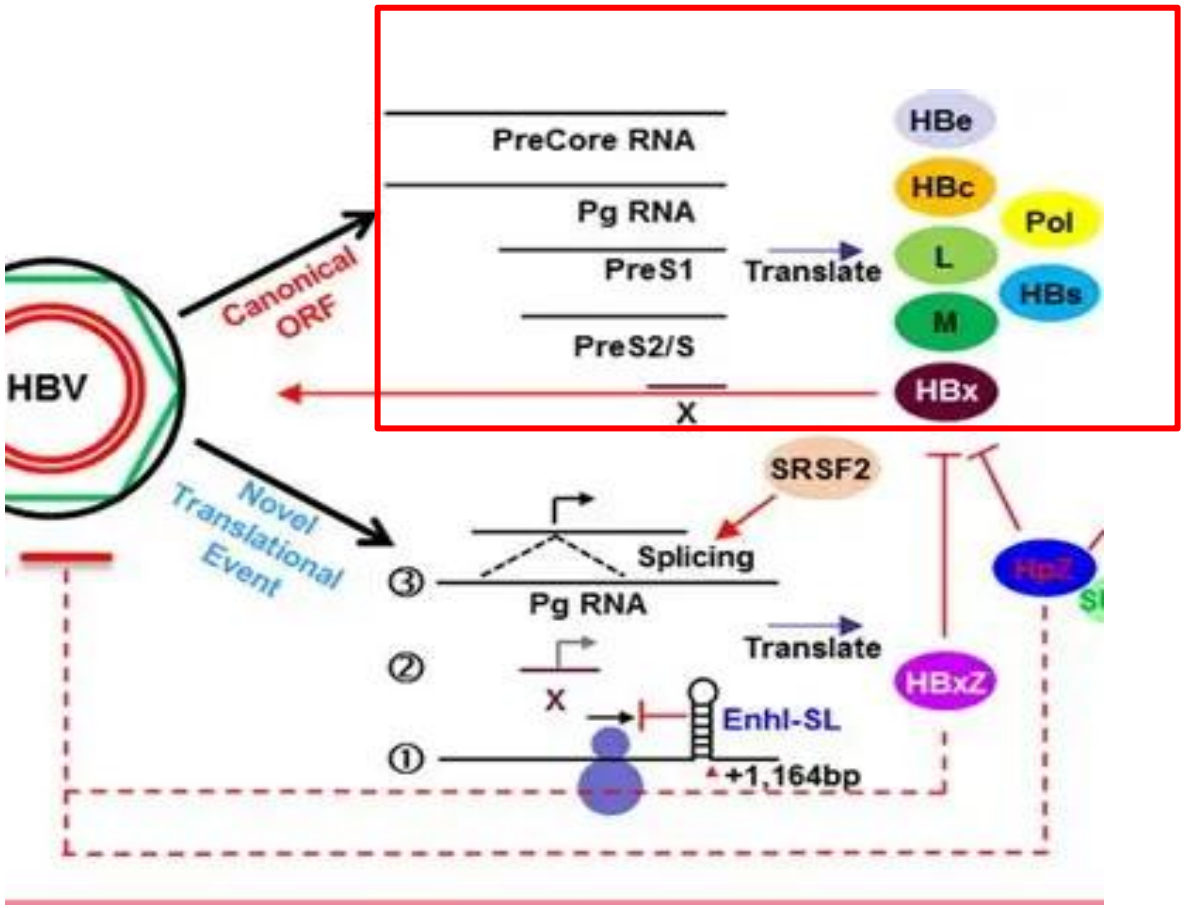


Steps in the cell where the HBV enters The HBV-NTCP-EGFR complex enters the cell mainly through reticulin-mediated endocytosis. After virus entry into the host hepatocytes, **a loose ring DNA (RC-DNA)** is delivered to the nucleus. Within the nuclei, the formation and assembly of nucleocapsids and the synthesis of **HBV cccDNA** are conducted through the following steps: the virus uses the host DNA-Taq polymerase along other enzymes to form cccDNA. Once formed, **cccDNA acts as a template for pregenomic RNA**



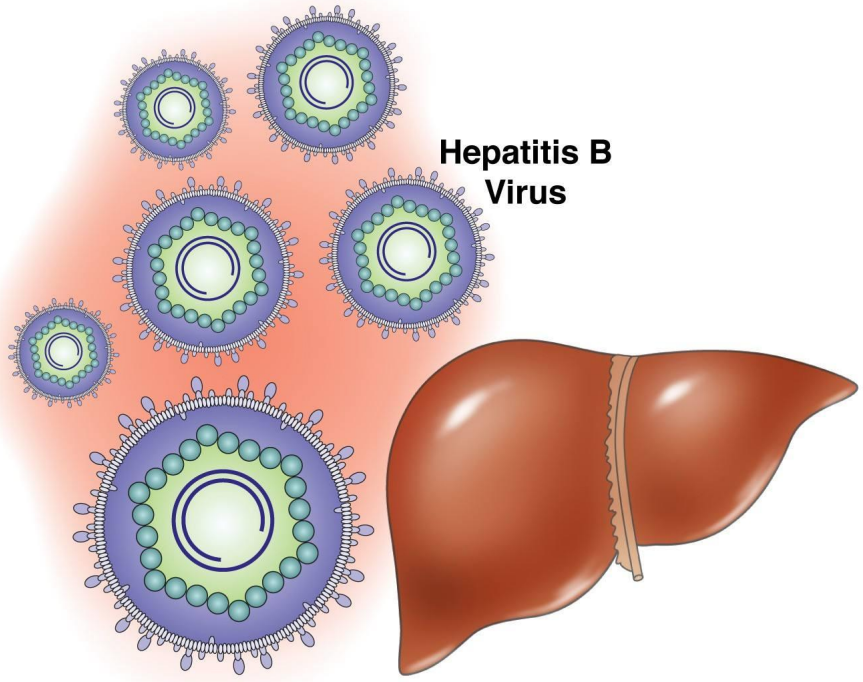
(pgRNA) and four diferent lengths of viral messenger RNA (mRNA) synthesis, by the action of host-cell RNA polymerase, thereby forming a reverse transcription template and a translation template. ① Reverse transcription template: Reverse transcription of HBV pregenomic RNA (pgRNA) forms negative singlestranded HBV DNA and then completes the synthesis of positive-stranded DNA. This step provides a template for HBV replication. ②Translation template: Four main messenger RNAs (MRNAs) encode viral proteins, including **hepatitis B surface antigens (HBsAg), e antigen (HBeAg), core antigens (HBcAg), HBV polymerase (Pol), and regulatory protein X (HBx) . It is important that HBcAg and HBx proteins regulate HBV DNA replication and HBsAg expression** through HBV cccDNA





During the HBV replication process, covalently closed circular DNA acts as a template for viral replication, transcribing five types of viral RNA with lengths of 3.5, 2.4, 2.1, and 0.7 kb, which are respectively translated into **HBeAg, core protein, polymerase (P) protein, HBsAg, and HBx protein.**

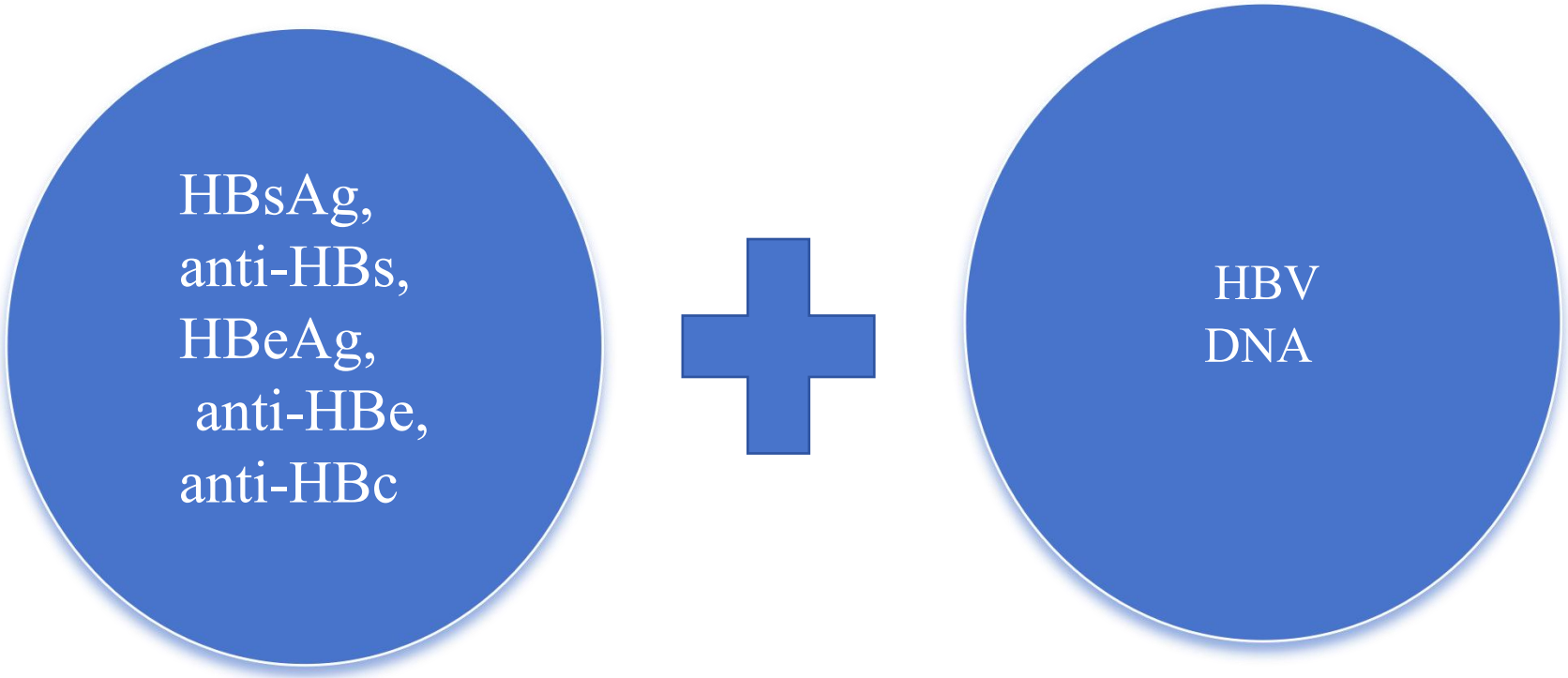
Structure of HBV viral particle





Healthy liver
Chronic hepatitis
Hepatitis and liver cirrhosis
Hepatocellular carcinoma

Key markers



Types of serological markers

Antigen-antibody		Nucleic acid
Antigen	Antibody	
HBsAg (Hepatitis B surface Antigen)	Anti-HBs (HB surface antibody)	HBV DNA (qualitative or quantitative)
HBcAg (Hepatitis B core Antigen) Does not appears in blood	IgM anti-HBc (HB core antibody IgM)	
	Total anti-HBc (IgM plus IgG)	
HBeAg (Hepatitis B e Antigen)	Anti-HBe (Hepatitis B e antibody)	

This table shows types of serological markers:

- HBsAg means Hepatitis B surface antigen, Anti-HBs means HB surface antibody

- HBcAg means Hepatitis B core antigen, IgM anti-HBc means HB core antibody IgM - Total anti-HBc means IgM and IgG

- HBeAg means Hepatitis B envelope antigen, Anti-HBe means Hepatitis B envelope antibody.

Hepatitis B surface antigen and antibody

HBsAg	• First marker to appear following HBV infection • Positivity indicates presence of virus in a person's body • Acute infection: Disappears within 6 months • Chronic infection: Persists for several years (lifelong in most) • Measurement of HBsAg concentration is being tried as a potential alternative marker of viremia and to monitor response to treatment, but still not well accepted
Anti-HBs	• Antibody to HBsAg • Is a neutralizing antibody and confers protection from infection • Appears following clearance of acute infection • Does not develop in those who have chronic infection • Also develops in response to hepatitis B vaccine • Presence indicates immunity following acute infection or vaccination • Anti-HBs titre >10 mIU/mL is considered to be protective • Persists for several years (often lifelong) after infection, but disappears in a few years after immunization

Hepatitis B core antigen and antibody

Test	Clinical interpretation
HBcAg	• An internal component of the virus • Present in the nucleus of infected cells • But, does not appears in infected person's blood • **Not tested in clinical settings** • Hepatitis B vaccine does not contain this antigen
Anti-HBc	• Develops in all those who get HBV infection, whether acute or chronic • Does not develop after immunization • Two types IgM and IgG
IgM anti-HBc	• Appears following acute infection, and persists for up to ~6 months • Hence: presence indicates recent (acute) infection • Occasionally, detectable (in low amount) during severe exacerbation of chronic infection
IgG (or Total) anti-HBc	• Develops soon after IgM anti-HBc • Most constant marker of exposure (current or past infection) • Positive total anti-HBc (IgG, IgM) with negative IgM anti-HBc in HBsAg negative indicates resolved infection

Hepatitis B e-antigen and antibody

HBeAg	• Produced in cells where virus is actively replicating, and is secreted into the plasma • Usually its presence indicates high viral load and high infectivity • Its absence indicates lower viral load, lower HBV DNA level. But, in some, may be absent despite high viral load (due to viral mutation) • Associated with high risk of HBV transmission following exposure, such as needle-stick injury, mother-to-child transmission, etc
Anti-HBe	• Indicates host immune response against HBeAg • Usually associated with reduced viral replication, lower HBV DNA titer and reduced infectivity • But also present in those in HBeAg-negative viral mutation
HBV DNA	• Direct and accurate marker of HBV replication • Serum level seems to correlate with the risk of disease progression • Used to decide need for anti-viral drugs • Also used to monitor efficacy of anti-viral drug treatment • Unit: almost 5 copies = 1 IU

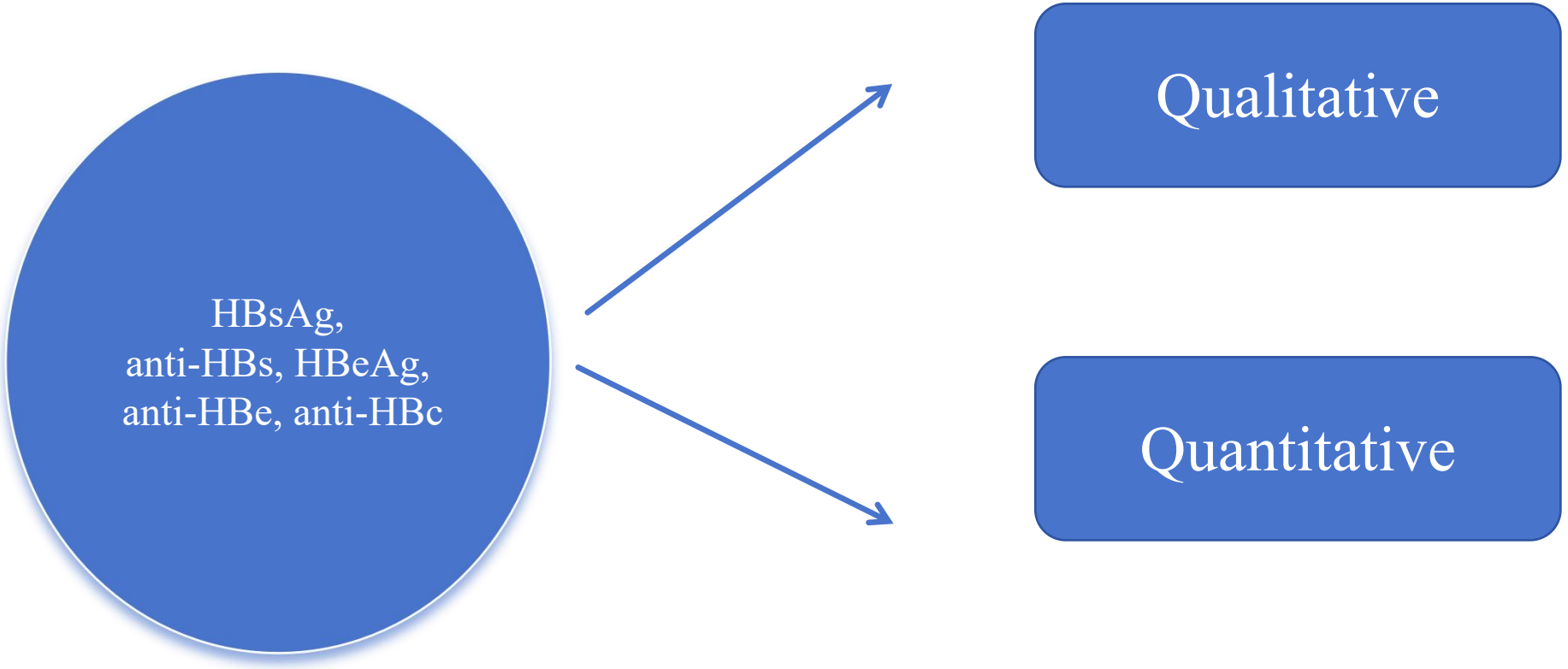
Classroom exercise

1. Learning to draw the HBV structure diagram

2. What are the serological markers of HBV?Please give an example.

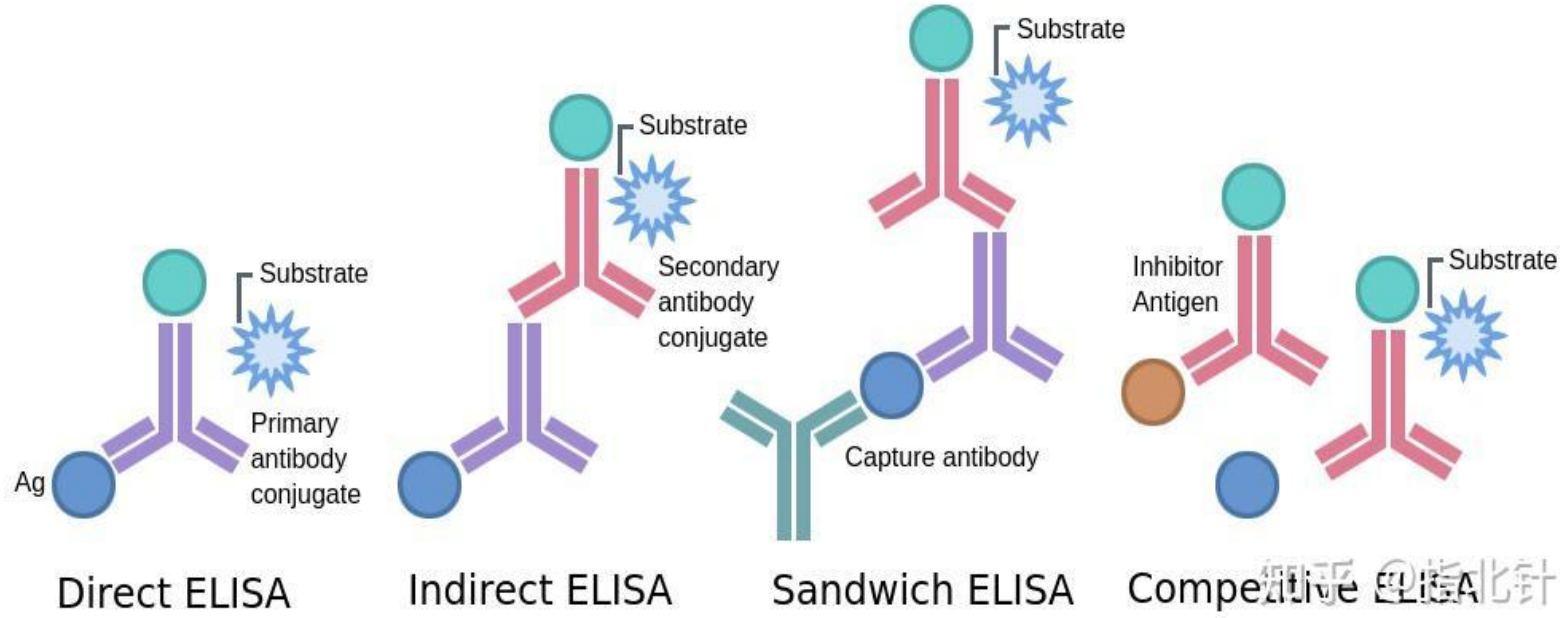
The second lesson

the testing approach in HBV

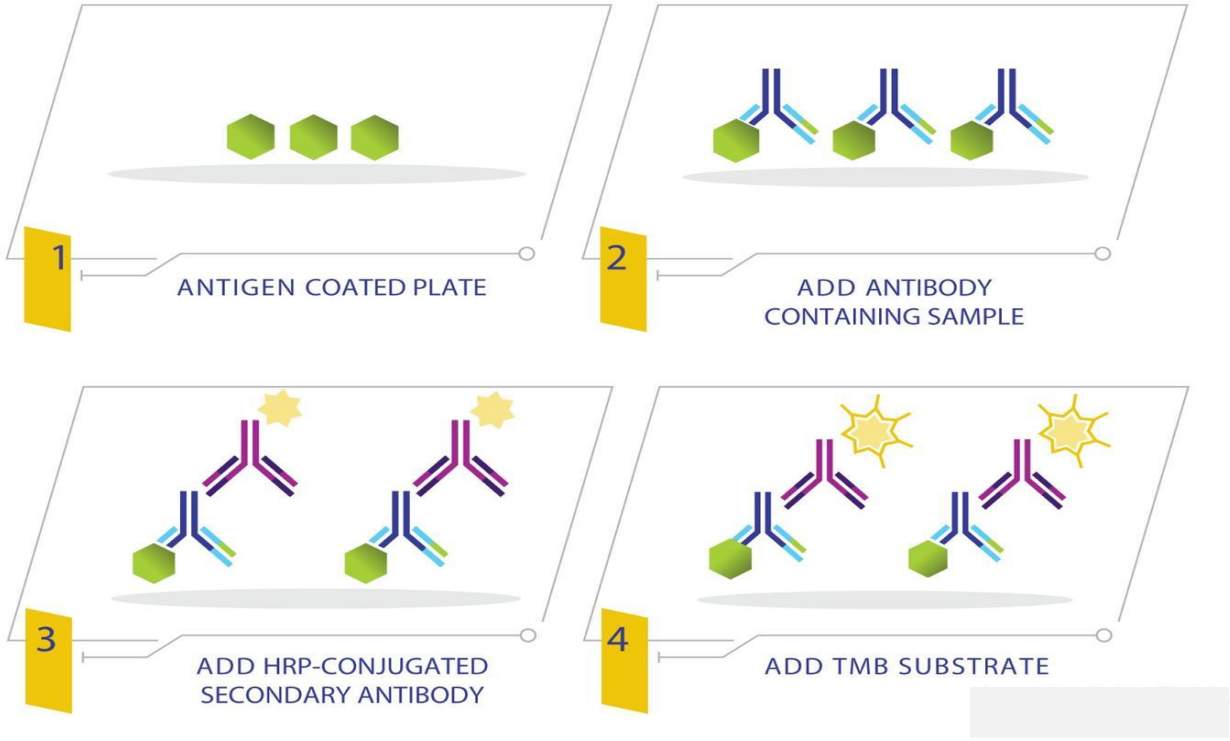

HBsAg,
anti-HBs, HBeAg,
anti-HBe, anti-HBc
Qualitative
Quantitative

Enzyme-Linked ImmunosorbentAssays-ELISA

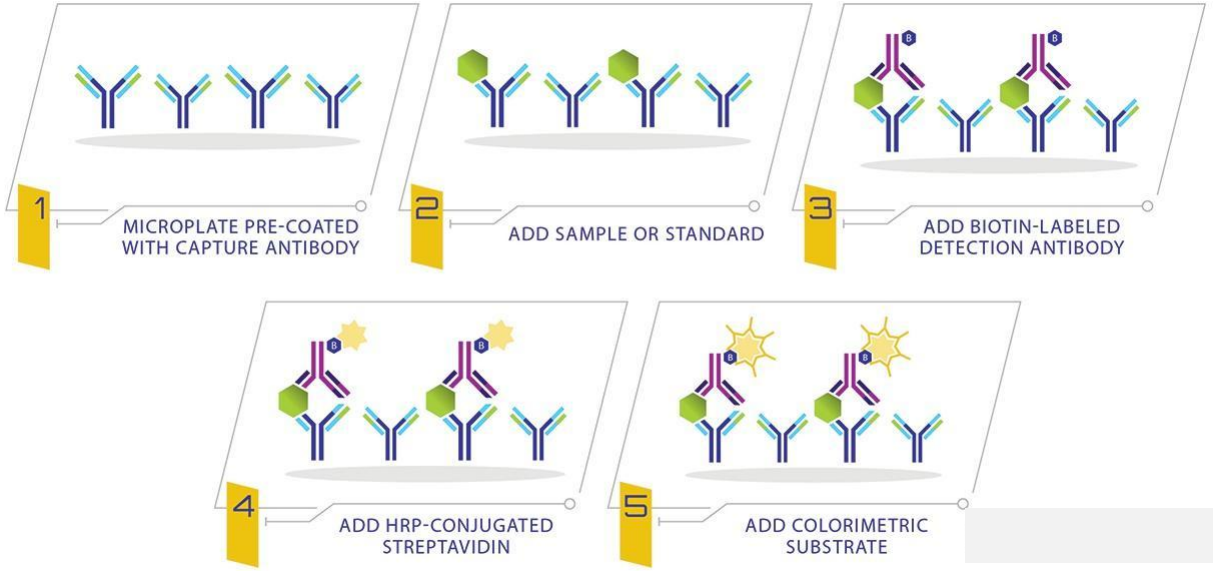
Enzyme-Linked Immunosorbent Assay (ELISA) is a laboratory technique widely used in immunology and molecular biology, first described by Eva Engvall and Peter Perlmann in 1971. The assay relies on the principle of antigen-antibody interactions and uses enzymes and colorimetric detection to quantify target molecules. It is mainly used to detect and measure the presence of specific proteins, peptides, antibodies, or antigens in biological samples. ELISA assays are commonly applied in various fields, including clinical diagnostics, drug research, and basic life science research.



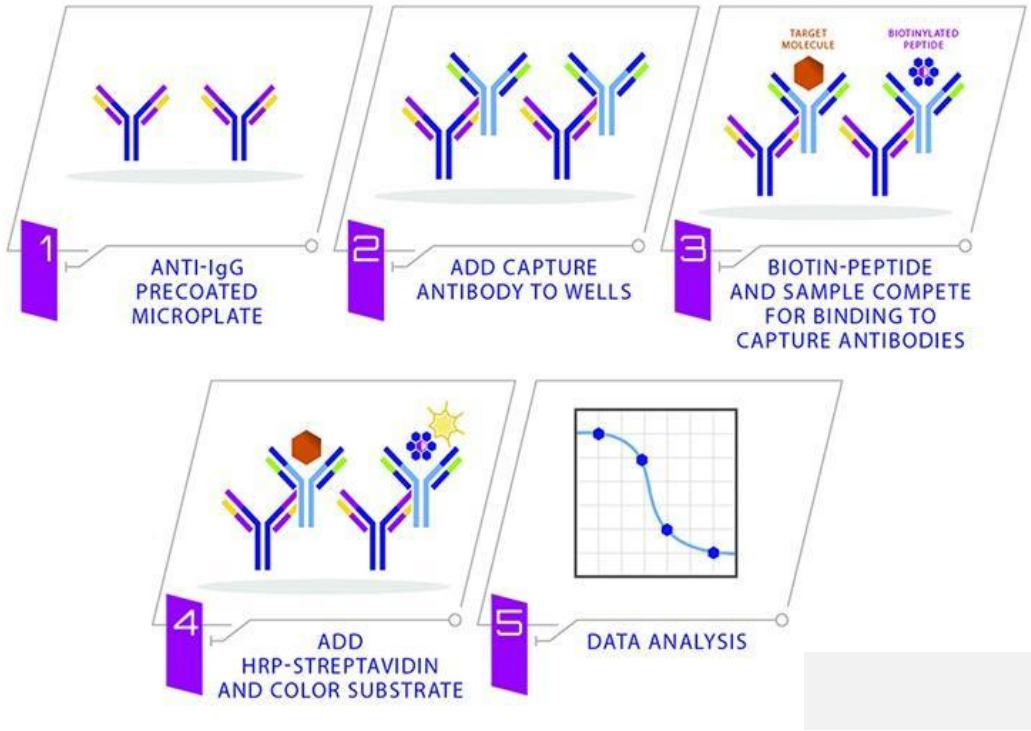
Indirect ELISA: Indirect ELISA is based on direct ELISA with the addition of an enzyme-labeled secondary antibody for detection. The antigen passively adheres to the wells through incubation. After washing, the antigen-specific antibody (primary antibody) is incubated with the antigen. The wells are washed, and the bound antibody is detected by adding a secondary antibody covalently linked to an enzyme. The secondary antibody is specific to the species that produced the primary antibody added.

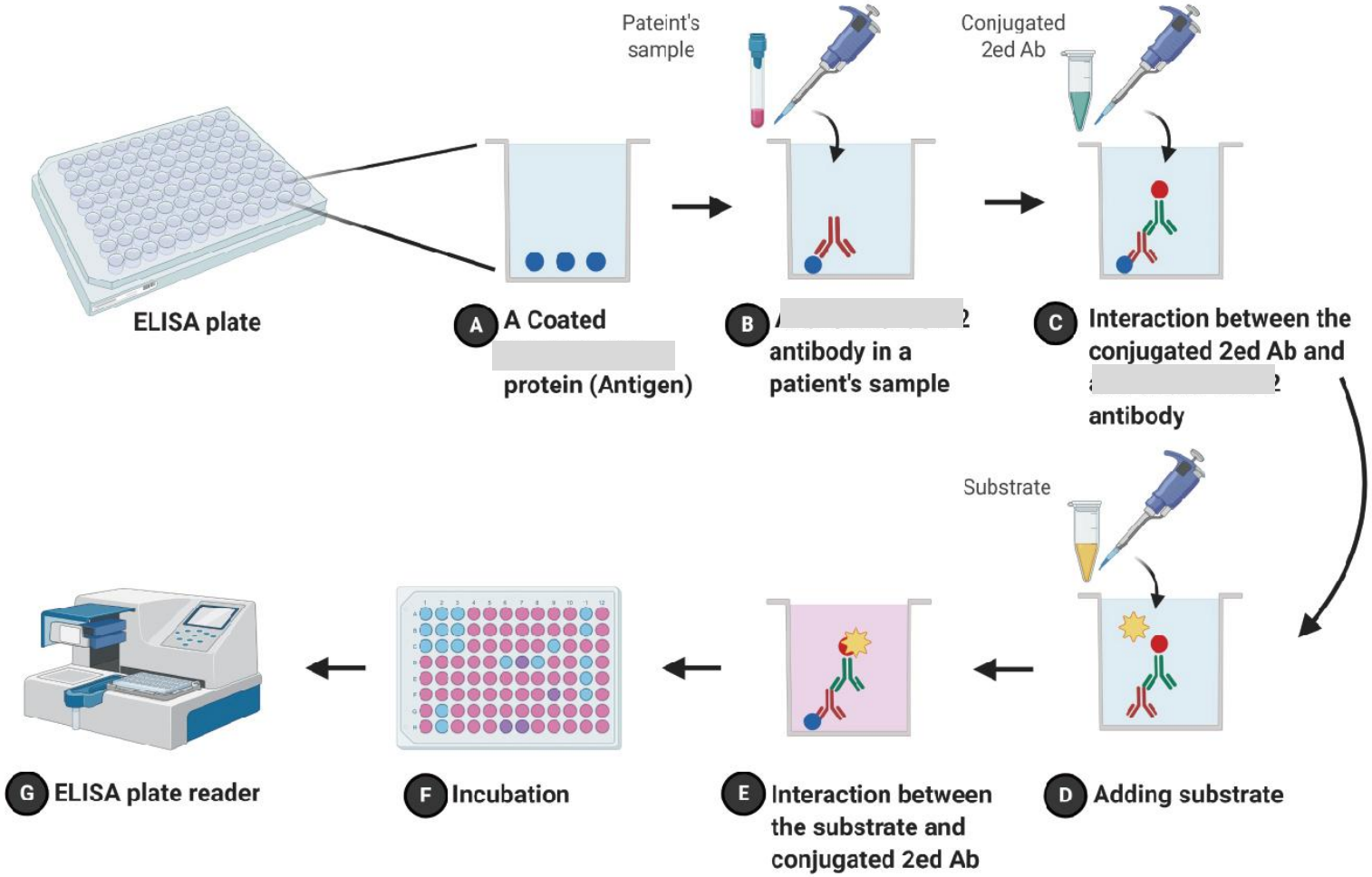


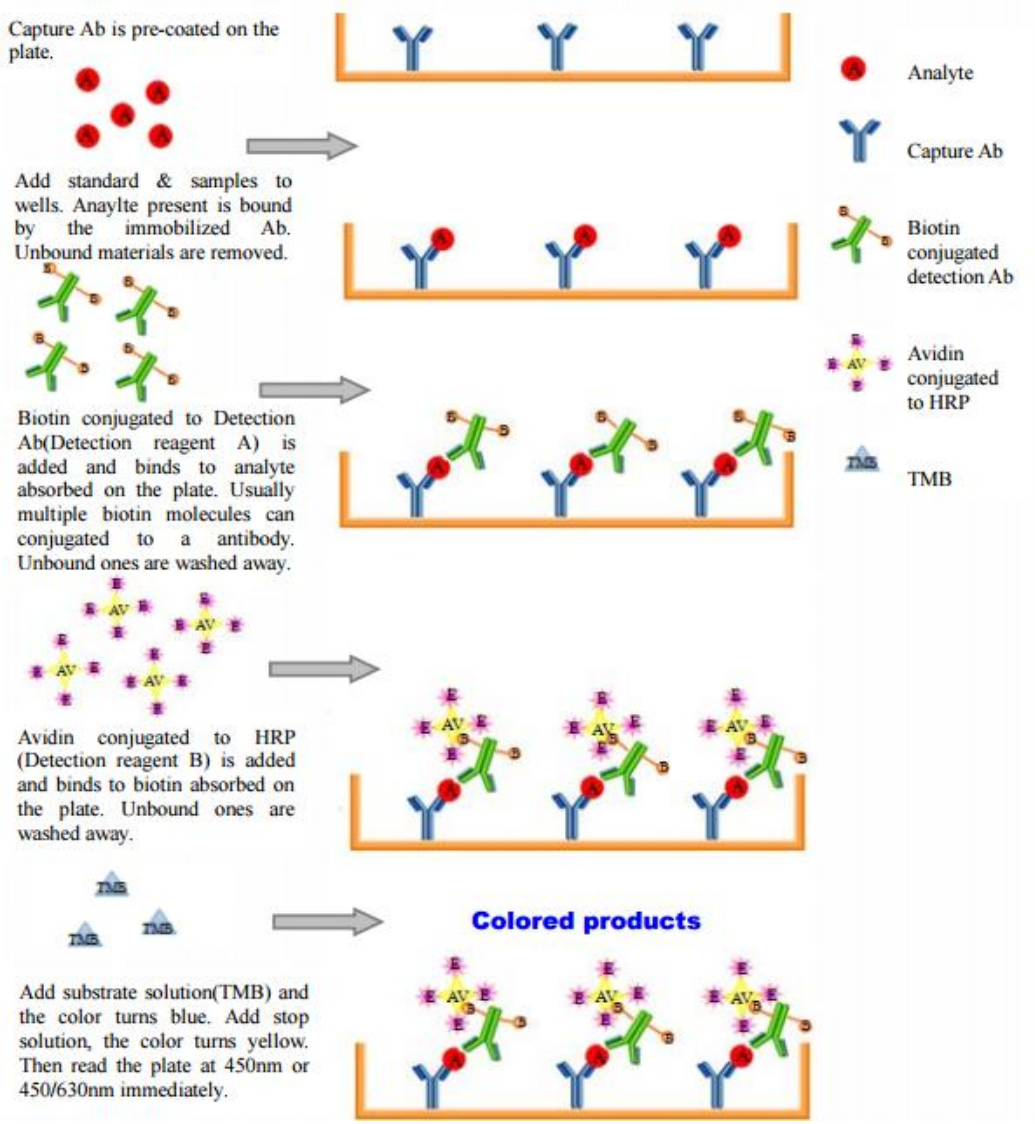
Sandwich ELISA: Attach the antibody that captures the antigen (capture antibody) to a solid-phase carrier. After washing away excess unbound antibodies, add the antigen to be specifically captured, and detect the antigen using either direct or indirect ELISA. This combination further enhances detection sensitivity and specificity, making it the most common form of ELISA experiment.



Competitive ELISA: In competitive ELISA, a fixed amount of labeled antigen competes with the sample antigen for binding to the immobilized antibody. The signal is inversely proportional to the concentration of antigen in the sample.




Pateint's sample
Conjugated 2ed Ab
ELISA plate
A A Coated protein (Antigen)
B antibody in a patient's sample
C Interaction between the conjugated 2ed Ab and antibody
Substrate
D Adding substrate
E Interaction between the substrate and conjugated 2ed Ab
F Incubation
G ELISA plate reader


Capture Ab is pre-coated on the plate.
Add standard & samples to wells. Anaylte present is bound by the immobilized Ab. Unbound materials are removed.
Biotin conjugated to Detection Ab(Detection reagent A) is added and binds to analyte absorbed on the plate. Usually multiple biotin molecules can conjugated to a antibody. Unbound ones are washed away.
Avidin conjugated to HRP (Detection reagent B) is added and binds to biotin absorbed on the plate. Unbound ones are washed away.
Add substrate solution(TMB) and the color turns blue. Add stop solution, the color turns yellow. Then read the plate at 450nm or 450/630nm immediately.
Colored products
Analyte
Capture Ab
Biotin conjugated detection Ab
Avidin conjugated to HRP
TMB

Classroom exercise

1. What are the types of Elisa?
2. Please draw a simple diagram for each type of ELISA method.

Polymerase Chain Reaction-PCR

1.Polymerase chain reaction (PCR) is a molecular biology technique used to amplify specific DNA fragments in vitro. Its core principle is based on the semi-conservative replication mechanism of DNA, rapidly amplifying DNA fragments in vitro using specific enzymes. Since PCR was first conceptualized by Kary Mullis in 1983 and officially invented in 1985, it has become a key tool in fields such as gene cloning, disease diagnosis, and forensic identification.

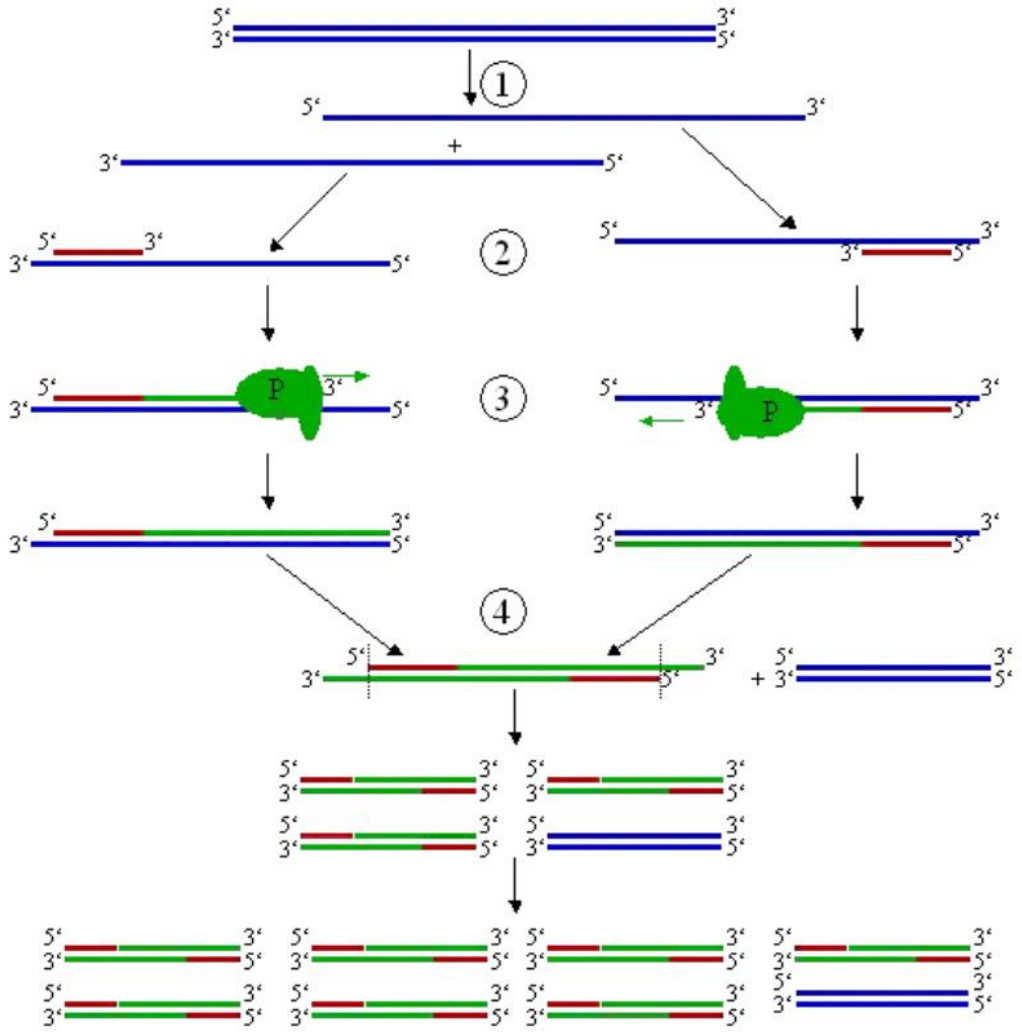
2. The Development History of PCR Technology

Early Conception: In 1971, Kjell Kleppe and Gobind Khorana first proposed the idea of in vitro amplification of nucleic acids, but due to technological limitations at the time, this idea could not be realized.

Invention and Breakthrough: In 1985, Kary Mullis invented PCR technology and obtained a patent for it in 1987. In 1988, the discovery of Taq DNA polymerase enabled PCR technology to be automated.

Commercialization and Promotion: In 1988, Taq enzyme was commercially promoted, and the subsequent appearance of PCR machines further facilitated the widespread use of PCR technology.

Technological Improvements: In 1991, the discovery of Pfu DNA polymerase further improved the accuracy and reliability of PCR. In 2003, the introduction of Phusion DNA polymerase marked the advent of a new generation of PCR technology.


5'
3'
1
2
3
4
P
+

3. The Principle of PCR Technology

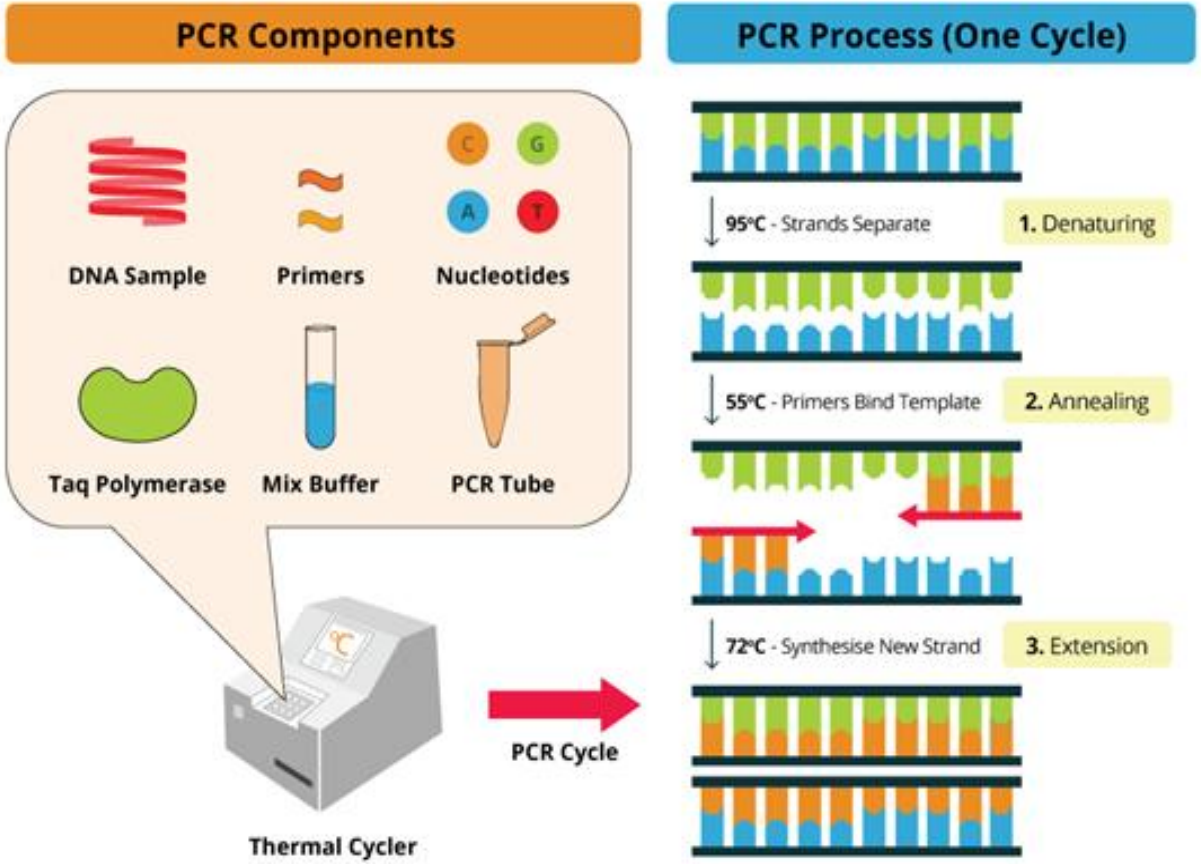
The PCR process mainly consists of three basic reaction steps:

Denaturation: The template DNA is heated to around 93°C for a certain period of time, causing the hydrogen bonds of the double-stranded DNA to break, resulting in separation into single strands.

Annealing: When the temperature is lowered to about 55°C, the primers pair and bind with the complementary sequences of the single-stranded template DNA.

Extension: At around 72°C, DNA polymerase extends along the template strand, gradually synthesizing a new DNA strand from the 3' end of the primer.

By repeatedly cycling through denaturation, annealing, and extension, more "semi-conservative replicated strands" can be obtained, and these new strands can serve as templates for the next cycle. Each cycle takes 2-4 minutes, and within 2-3 hours, the target gene can be amplified millions of times.


PCR Components
PCR Process (One Cycle)
DNA Sample
Primers
Nucleotides
C
G
A
T
Taq Polymerase
Mix Buffer
PCR Tube
Thermal Cycler
PCR Cycle
95°C - Strands Separate
1. Denaturing
55°C - Primers Bind Template
2. Annealing
72°C - Synthesise New Strand
3. Extension

Classroom exercise

1. What are the steps of PCR?

2. Please briefly draw the PCR process .

The Third lesson

Clinical Significance of HBV serologic marker

HBsAg (Hepatitis B Surface Antigen)

Clinical significance: Marker of active infection

Detection: Appears 1-10 weeks post-exposure

Persistence: >6 months = chronic infection

Clearance: Indicates recovery or treatment success

Anti-HBs (Hepatitis B Surface Antibody)

Protective antibody: Neutralizing immunity

Sources: Vaccination or natural infection recovery

Titer: >10 mIU/mL = protective

Clinical use: Vaccine response assessment

HBeAg (Hepatitis B e Antigen)

Viral replication: Marker of active replication

Infectivity: High transmission risk

Seroconversion: HBeAg to anti-HBe = reduced replication

Treatment target: HBeAg seroconversion

Anti-HBe (Hepatitis B e Antibody)

Appearance: After HBeAg clearance

Significance: Reduced viral replication

Caveat: Precore mutants may remain HBeAg-negative

Anti-HBc (Hepatitis B Core Antibody)

Total anti-HBc: Lifetime marker of infection

IgM anti-HBc: Acute infection (first 6 months)

IgG anti-HBc: Past or chronic infection

Window period: Only marker in some cases

HBV DNA Quantification

Direct measurement: Viral load assessment

Units: IU/mL or copies/mL

Clinical applications:

Treatment indication

Monitoring response

Resistance detection

Prognosis assessment

Interpretation Patterns - Acute Infection
Early phase: HBsAg+, HBeAg+, HBV DNA high
Window period: HBsAg-, anti-HBc IgM+
Recovery: Anti-HBs+, anti-HBc IgG+
Chronic: HBsAg+ >6 months

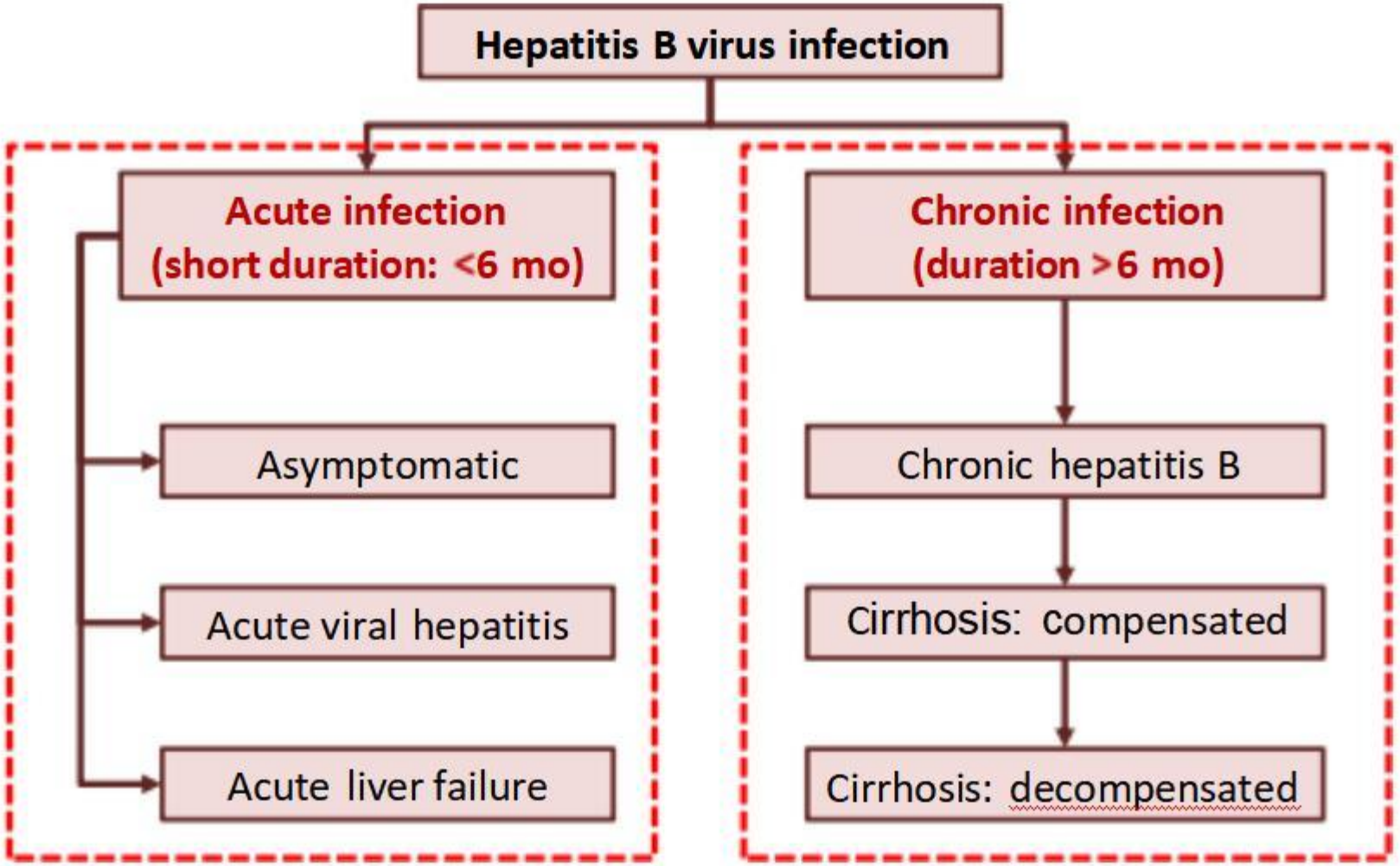
The Application of HBV Markers in the Staging of the Natural History of Chronic HBV Infection

Chronic HBV infection can be artificially divided into four stages: the immune tolerant phase, the immune clearance phase, the immune control phase, and the reactivation phase. Viral markers show different combinations of results at different stages.

Qualitative/quantitative marker test results corresponding to different stages of the natural history of chronic HBV infection

HBV marker	the immune tolerant phase	the immune clearance phase	the immune control phase	the reactivation phase
HBsAg	>1×104	positive	<1×103	positive
Anti-HBs	negative	negative	negative	negative
HBeAg	positive	positive	negative	negative
Anti-HBe	negative	negative	positive	positive
Anti-HBc quantitative(IU/ml)	低水平（<103）	高水平（<104）	较低水平（103～104）	Rise
HBV DNA(IU/ml)	>2×107	positive	negative	positive
HBV pgRNA(copies/ml)	>2×107	>2×104	<2×103	≥2×103
HBcrAg(U/ml)	>2×107	>2×104	<2×103	≥2×103

Consequences of hepatitis B virus infection



The consequences of hepatitis B virus infection are divided into two clinical courses:

After hepatitis B virus infection, the individual may have acute infection, which is defined as infection duration less than 6 months, OR chronic infection, where the infection duration lasts more than 6 months.

- In acute infection, the patient may be/have:

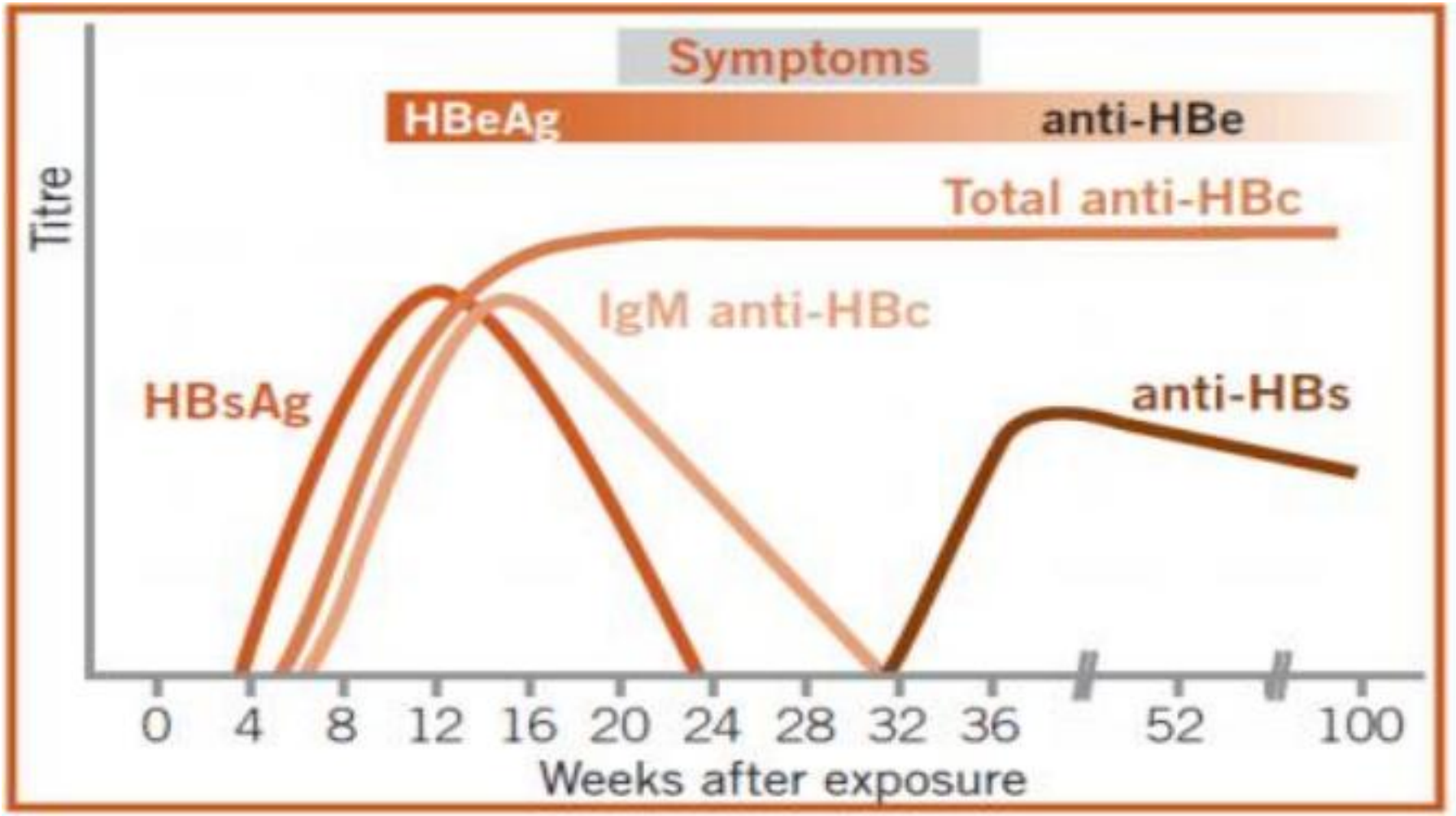
(a) Asymptomatic: the infected persons have no clinical symptoms and they do not notice the infection.

(b) Acute viral hepatitis: the infected persons have clinical symptoms such as general malaise, appetite loss or flu-like symptoms and usually they resolve with no treatment or only needing supportive care.

(c) In acute liver failure: the infected persons have severe clinical symptoms related to liver failure, such as jaundice, ascites and hepatic encephalopathy. In this stage, patients generally will not be able to recover without liver transplantation (i.e. mortality is high).

- In chronic infection, hepatitis B virus infection causes chronic hepatitis and the chronic inflammation over the next 20 – 30 years, after which may result in development of cirrhosis.

Serological pattern of acute HBV infection



This figure shows the serological pattern of acute HBV infection:

- After infection, first, HBsAg appears and increase within 2-10 weeks.

- Next, IgM anti-HBc and total anti-HBc increases after 2 weeks

- IgM anti-HBc is a specific marker for acute HBV infection and it decrease and disappears after 32 weeks.

- Total anti-HBc, mainly IgG anti-HBc continues to be positive for life. Thus, total anti- HBc is the marker for post-infection.

- HBsAg decrease and disappears within 6 months, with acute infection.

- After that, the neutralizing antibody, anti-HBs, appears. In this phase, the person is considered as cured.

Interpretation of serological markers

HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
-			-	Never exposed

We will now look at interpreting the panel of HBV serological markers.

This is important in clinical practice when you receive results back from the laboratory.

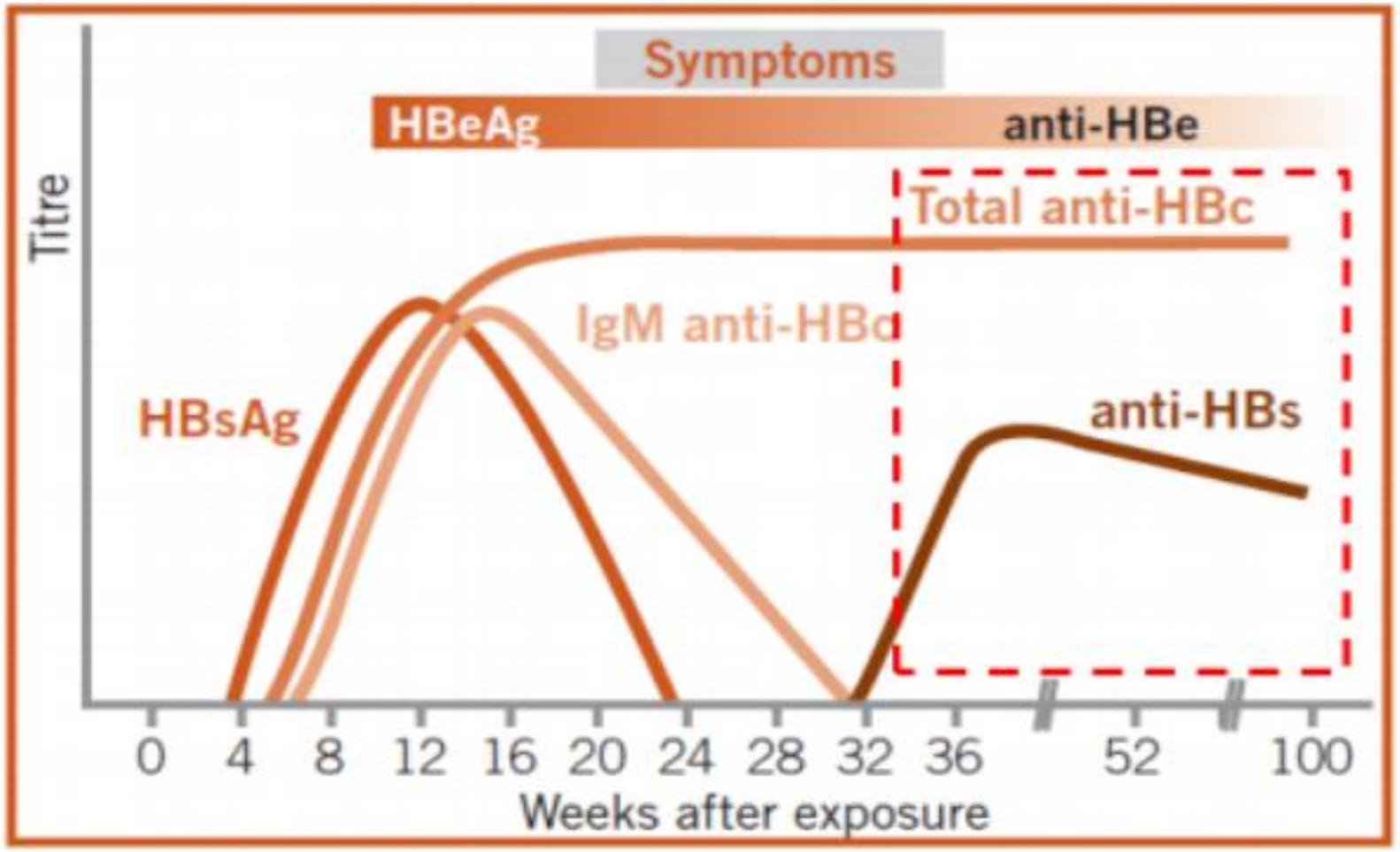
HBsAg negative and Anti-HBs negative means **“never exposed”.**

Interpretation of serological markers

HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
-	+	-	+	Past natural infection, cleared, immunity achieved

Total anti-HBC positive and anti-HBs positive means “past natural infection, cleared and immunity achieved”.

Serological pattern of acute HBV infection



In this figure, the red dash box shows “past natural infection, cleared and immunity achieved”

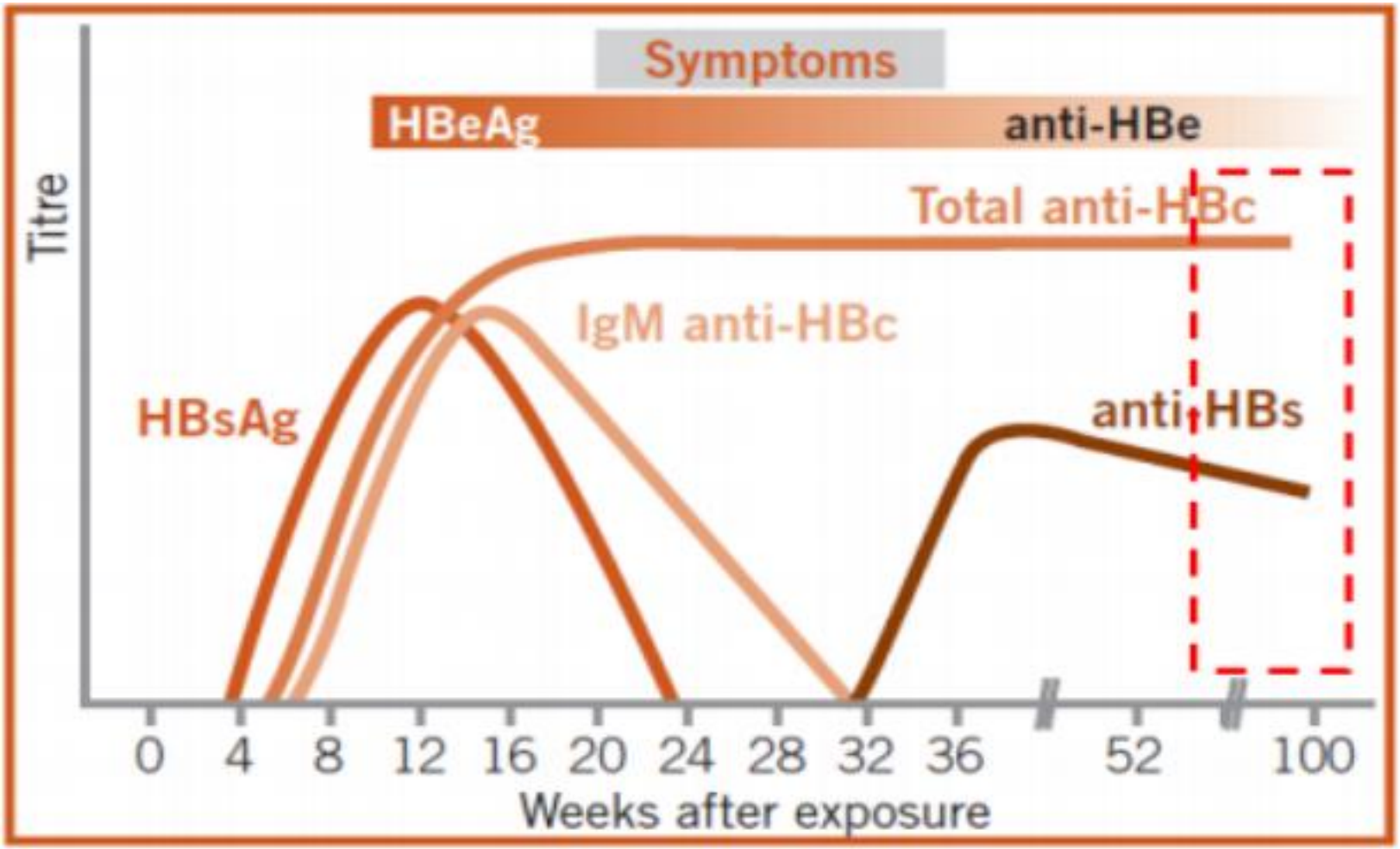
- where total anti-HBC and anti-HBs tests are positive

Interpretation of serological markers

HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
-	+	-	-	Past natural infection, cleared, anti-HBs has waned over time

Total anti-HBC only positive also means past natural infection, cleared and anti-HBs levels have waned over time.

Serological pattern of acute HBV infection



This figure illustrates waning of the anti-HBs levels, which have dropped and disappeared over time, and where total anti-HBC remains positive.

Interpretation of the test: “past natural infection, infection cleared and anti-HBs levels have waned over time”.

Interpretation of serological markers

HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
-	-	-	+	Immunity due to vaccination

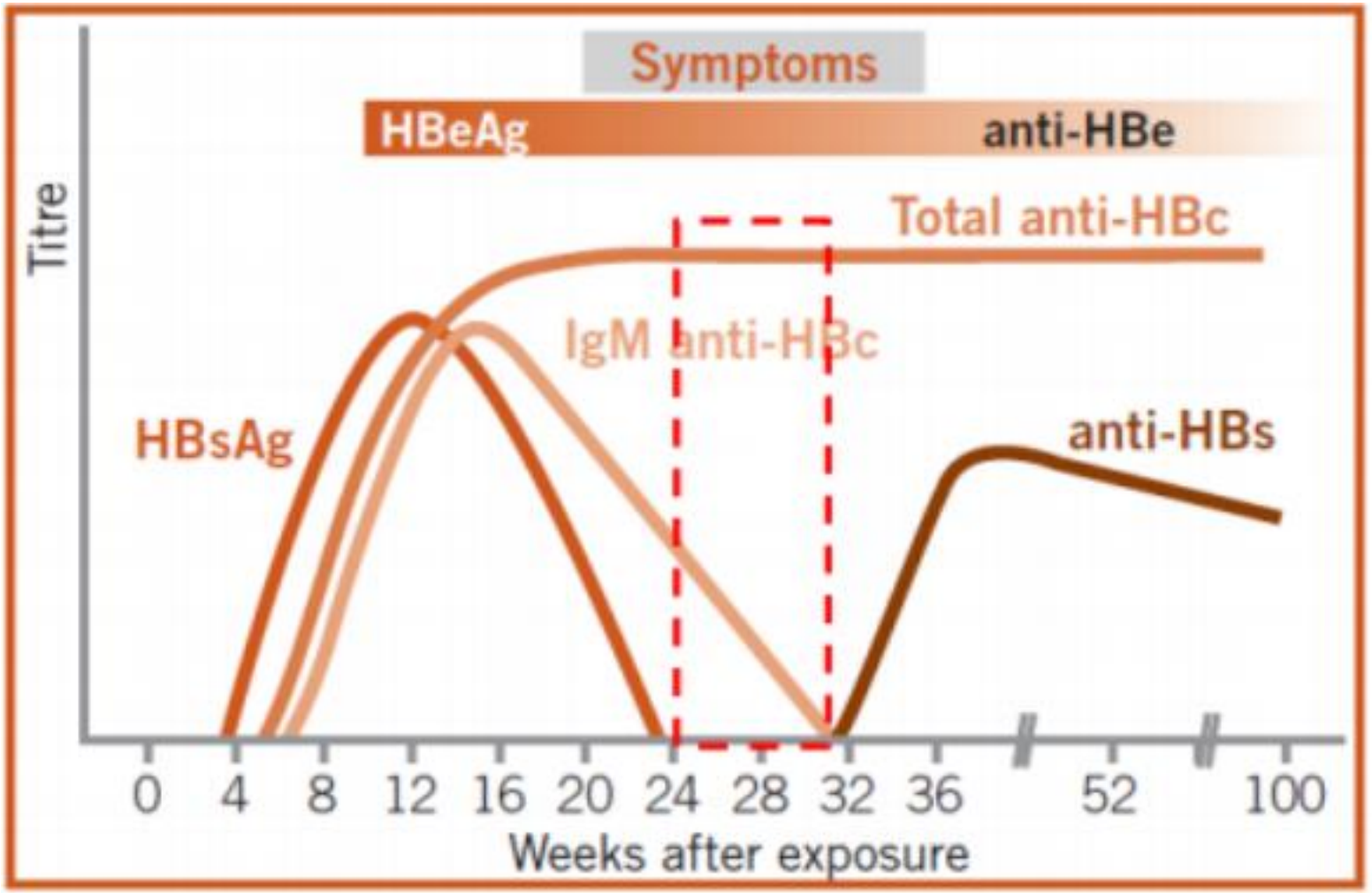
Anti-HBs only positive means “immunity due to vaccination”.

Interpretation of serological markers

HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
-	+	+	+	Recent infection, recovered, immunity achieved

HBs negative, total anti-HBc positive, IgM anti-HBc positive and anti-HBs positive means “recent infection, recovered, immunity achieved”.

Serological pattern of acute HBV infection



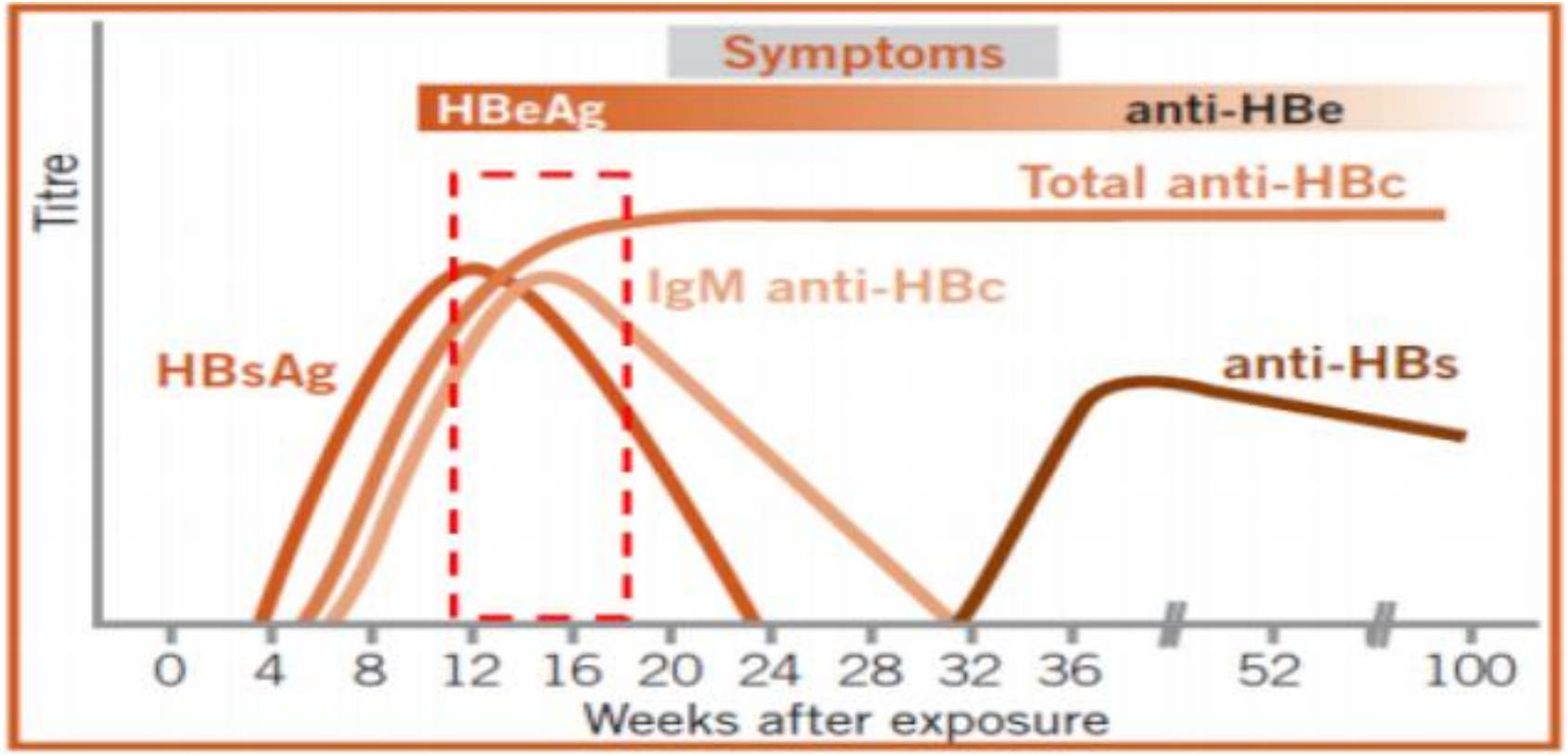
In this figure illustrate the above slide on “recent infection, recovered, immunity achieved”. Noted the red dash box: Anti-HBs levels have dropped and disappeared.

Interpretation of serological markers

HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
+	+	+	-	Acute infection, ongoing

HBs positive , total anti-HBc positive, IgM anti-HBC positive and anti-HBc negative means “acute infection, ongoing”.

Serological pattern of acute HBV infection



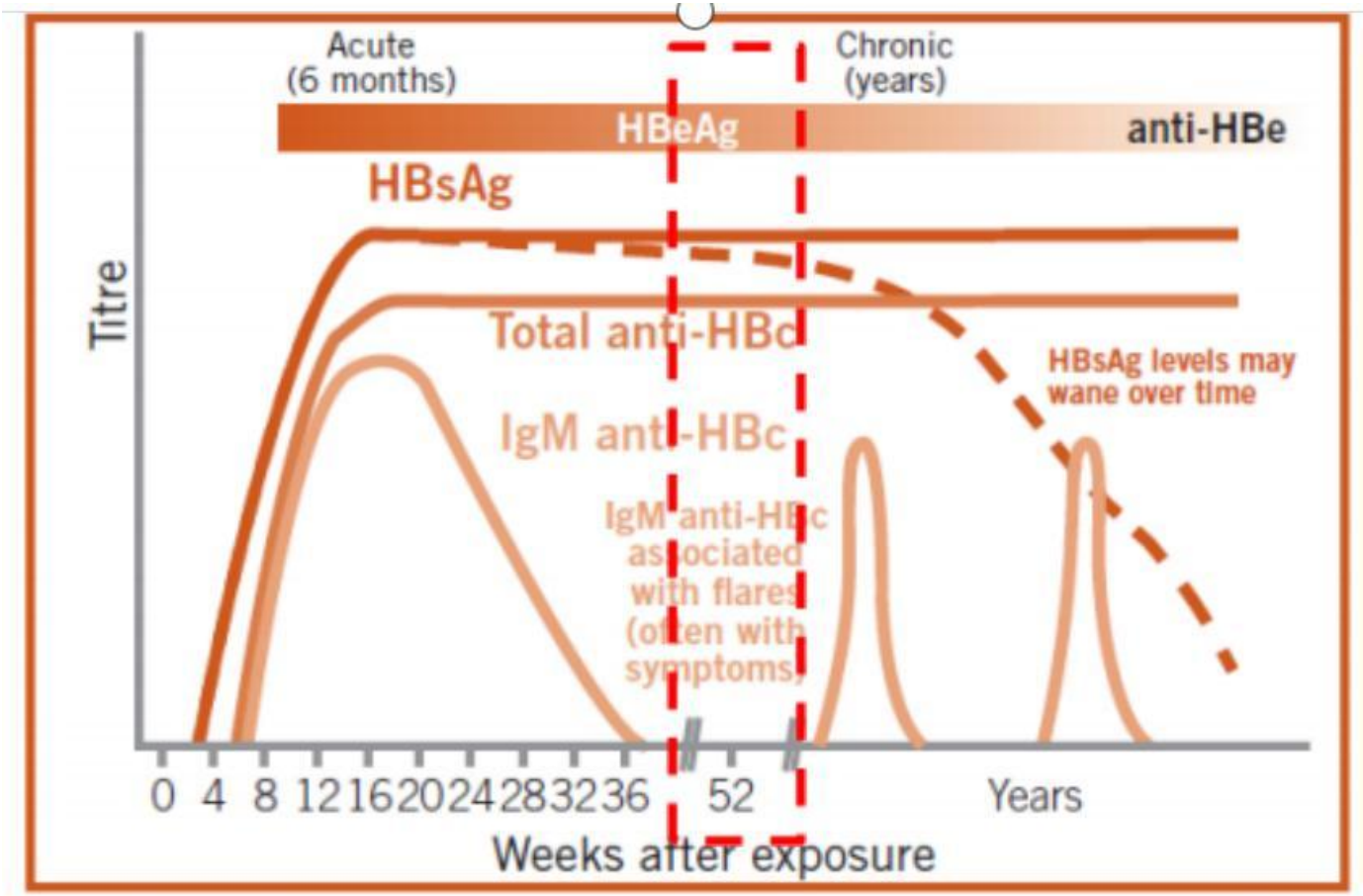
In this figure (red dash box) illustrates that
“acute infection, is ongoing”, and where HBs is positive ,
total anti-HBc is positive, IgM anti-HBC is positive and
anti-HBc is negative

Interpretation of serological markers

HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
+	+	-	-	Chronic infection (ongoing)

HBsAg positive, total anti-HBc positive, IgM anti-HBc negative and anti-HBs negative means chronic infection is ongoing.

Serological pattern of chronic HBV infection



Let's have a look at the serological pattern of CHRONIC infection

This is the part of the red dash box – where chronicity is being established, and the person moves into a chronic phase.

Interpretation of serological markers

HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
-			-	Never exposed
-	+	-	+	Past natural infection, cleared, immunity achieved
-	+	-	-	Past natural infection, cleared, anti-HBs has waned over time
-	-	-	+	Immunity due to vaccination
-	+	+	+	Recent infection, recovered, immunity achieved
+	+	+	-	Acute infection, ongoing
+	+	-	-	Chronic infection (ongoing)

This is summary table of interpretation of serological markers.

Take some time to understand this:

- From the point of screening for HBV infection, the lower two (boxed part) is important.

- If HBsAg is positive after screening,IgM anti-HBc is useful to differentiate between acute and chronic infection.However,IgM anti-HBc may not be affordable or available in resource-limiting settings.

- In such cases, clinical symptoms related to acute hepatitis or chronic infection are useful.

Classroom exercise

1.What are the stages of the natural history of chronic HBV infection?

2.What are their characteristics?



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DEPARTMENT OF LABORATORY MEDICINE

Thank you