

# **Training workshop on screening, diagnosis and treatment of hepatitis B and C**

## **Session 8**

### **Testing and serological markers of hepatitis B virus**

## Learning objectives

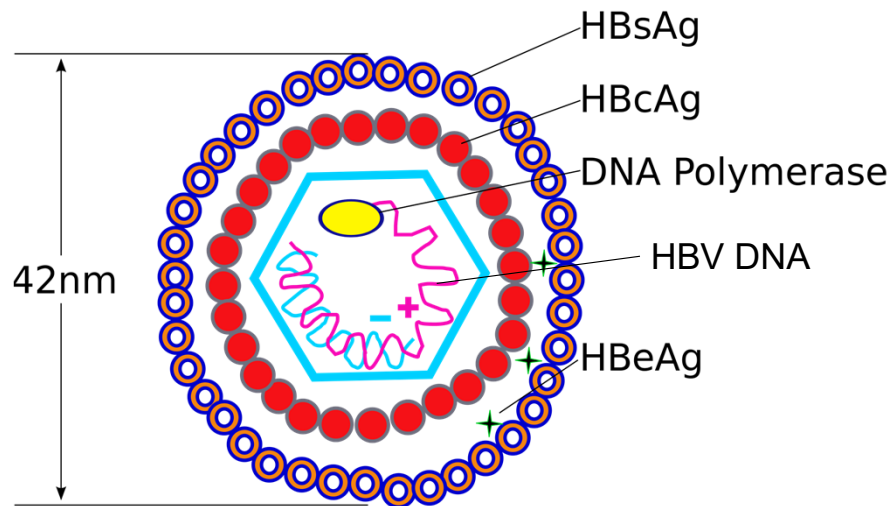
At the end of this session, the participants should:

- Know about various serological markers of HBV infection
- Understand the use of HBV markers in differentiating between various phases of HBV infection
- Understand the testing approach in HBV

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## Structure of HBV viral particle



This slide shows the structure of the HBV viral particle:

- HBsAg, HBV surface antigen is on surface of virus.
- There is nucleocapsid, core, in inside of viral particle.
- HBcAg, HBV core antigen is on surface of nucleocapsid.
- HBV DNA is inside of nucleocapsid.
- HBeAg, HBV envelope antigen, is located between HBV surface and core.

## 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.

## HBV serological markers

Test	Clinical interpretation
<b>HBsAg</b> (hepatitis B surface antigen)	<b>Hallmark of infection</b> Positive in the early phase of acute infection and persists in chronic infection Quantification of HBsAg is a potential alternative marker of viraemia and it is also used to monitor the response to antiviral treatment
<b>Anti-HBc IgM</b> (hepatitis B core antibody)	IgM subclass of anti-HBc and observed during <b>acute infection</b> (used to differentiate between acute and chronic HBV infection) Might become positive during severe exacerbation of chronic infection
<b>Anti-HBc (total)</b>	Develops around 3 months after infection (most constant marker of infection) Total anti-HBc (IgM, IgA and IgG) indicates <b>resolved infection</b>
<b>HBeAg</b> (hepatitis B e antigen)	Viral protein usually associated with <b>high viral load</b> and <b>high infectivity</b>
<b>Anti-HBe</b> (hepatitis B e antibody)	Antibody to HBeAg usually indicates decreasing HBV DNA But present in the immune-control and immune-escape phases
<b>Anti-HBs</b> (hepatitis B surface antibody)	Neutralizing antibody that confers protection from infection Recovery from acute infection (with anti-HBc IgG) Immunity from vaccination

Here, we introduce the various serological markers of HBV infection, which will help us to understand the various phases of acute and chronic hepatitis B.

HBsAg (hepatitis B surface antigen) is the hallmark of HBV infection.

Anti-HBc IgM (hepatitis B core antibody) is observed during acute infection.

Anti-HBc (total antibody against HBV core antigen) indicates the presence of IgM and/or IgG against the core antigen. A positive total anti-HBc with negative anti-HBc IgM antibodies indicates resolved infection.

HBeAg (hepatitis B envelope antigen) is viral protein associated usually with a high viral load and high infectivity.

Anti-HBe (antibody to HBeAg) usually indicates decreasing HBV DNA.

Anti-HBs is a neutralizing antibody.

## Hepatitis B surface antigen and antibody

Test	Clinical interpretation
HBsAg	<ul style="list-style-type: none"> <li>• First marker to appear following HBV infection</li> <li>• Positivity indicates presence of virus in a person's body</li> <li>• Acute infection: Disappears within 6 months</li> <li>• Chronic infection: Persists for several years (lifelong in most)</li> <li>• 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</li> </ul>
Anti-HBs	<ul style="list-style-type: none"> <li>• Antibody to HBsAg</li> <li>• Is a neutralizing antibody and confers protection from infection</li> <li>• Appears following clearance of acute infection</li> <li>• Does not develop in those who have chronic infection</li> <li>• Also develops in response to hepatitis B vaccine</li> <li>• Presence indicates immunity following acute infection or vaccination</li> <li>• Anti-HBs titre &gt;10 mIU/mL is considered to be protective</li> <li>• Persists for several years (often lifelong) after infection, but disappears in a few years after immunization</li> </ul>

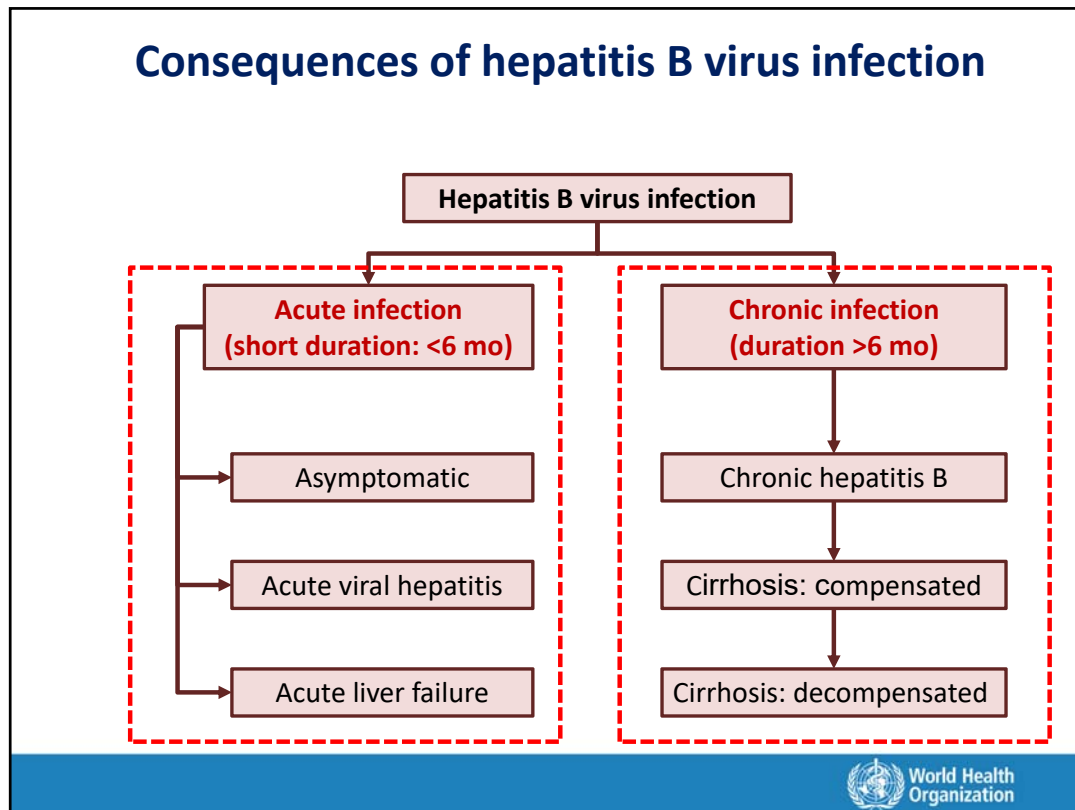
## Hepatitis B core antigen and antibody

Test	Clinical interpretation
HBcAg	<ul style="list-style-type: none"> <li>• An internal component of the virus</li> <li>• Present in the nucleus of infected cells</li> <li>• But, does not appear in infected person's blood</li> <li>• <b><u>Not tested in clinical settings</u></b></li> <li>• Hepatitis B vaccine does not contain this antigen</li> </ul>
Anti-HBc	<ul style="list-style-type: none"> <li>• Develops in all those who get HBV infection, whether acute or chronic</li> <li>• Does not develop after immunization</li> <li>• Two types IgM and IgG</li> </ul>
IgM anti-HBc	<ul style="list-style-type: none"> <li>• Appears following acute infection, and persists for up to ~6 months</li> <li>• Hence: presence indicates recent (acute) infection</li> <li>• Occasionally, detectable (in low amount) during severe exacerbation of chronic infection</li> </ul>
IgG (or Total) anti-HBc	<ul style="list-style-type: none"> <li>• Develops soon after IgM anti-HBc</li> <li>• Most constant marker of exposure (current or past infection)</li> <li>• Positive total anti-HBc (IgG, IgM) with negative IgM anti-HBc in HBsAg negative indicates resolved infection</li> </ul>



## Hepatitis B e-antigen and antibody

Test	Clinical interpretation
HBeAg	<ul style="list-style-type: none"> <li>Produced in cells where virus is actively replicating, and is secreted into the plasma</li> <li>Usually its presence indicates high viral load and high infectivity</li> <li>Its absence indicates lower viral load, lower HBV DNA level. But, in some, may be absent despite high viral load (due to viral mutation)</li> <li>Associated with high risk of HBV transmission following exposure, such as needle-stick injury, mother-to-child transmission, etc</li> </ul>
Anti-HBe	<ul style="list-style-type: none"> <li>Indicates host immune response against HBeAg</li> <li>Usually associated with reduced viral replication, lower HBV DNA titer and reduced infectivity</li> <li>But also present in those in HBeAg-negative viral mutation</li> </ul>
HBV DNA	<ul style="list-style-type: none"> <li>Direct and accurate marker of HBV replication</li> <li>Serum level seems to correlate with the risk of disease progression</li> <li>Used to decide need for anti-viral drugs</li> <li>Also used to monitor efficacy of anti-viral drug treatment</li> <li>Unit: almost 5 copies = 1 IU</li> </ul>



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:

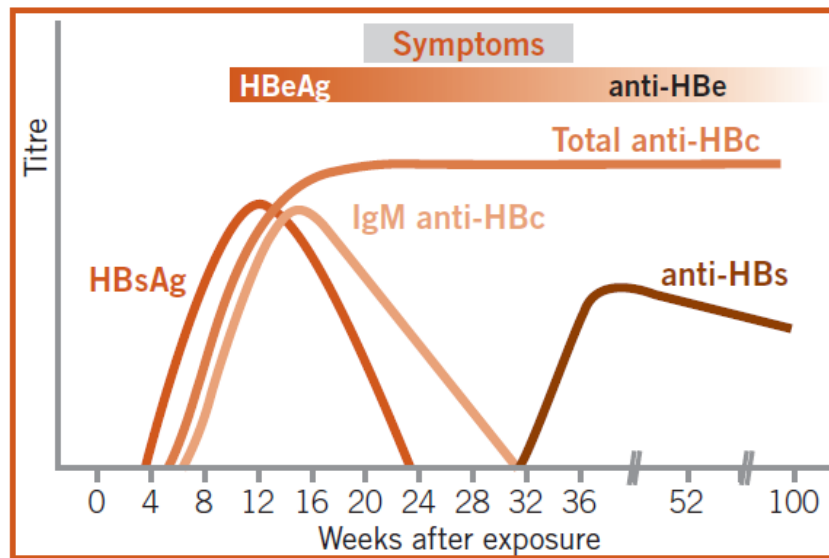
- (a) Asymptomatic: the infected persons have no clinical symptoms and they do not notice the infection.

- (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



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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.

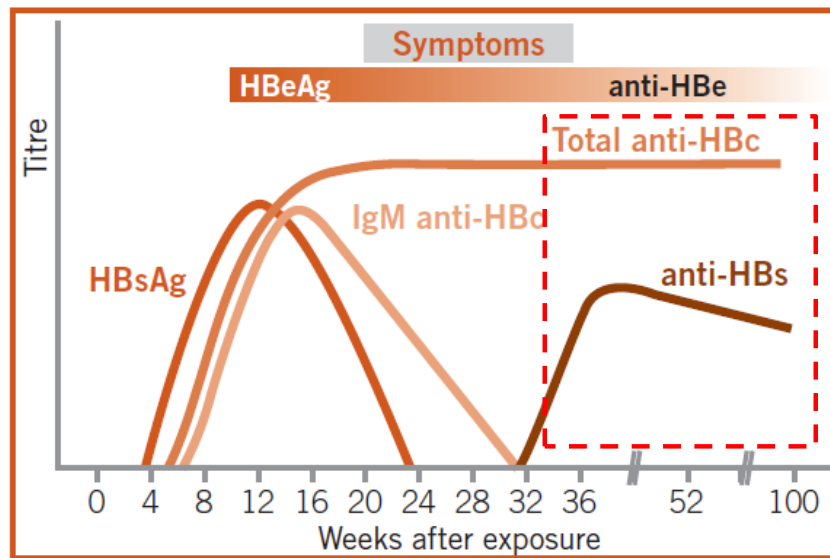
HBsAg negative and Anti-HBs negative means “never exposed”.

## 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



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

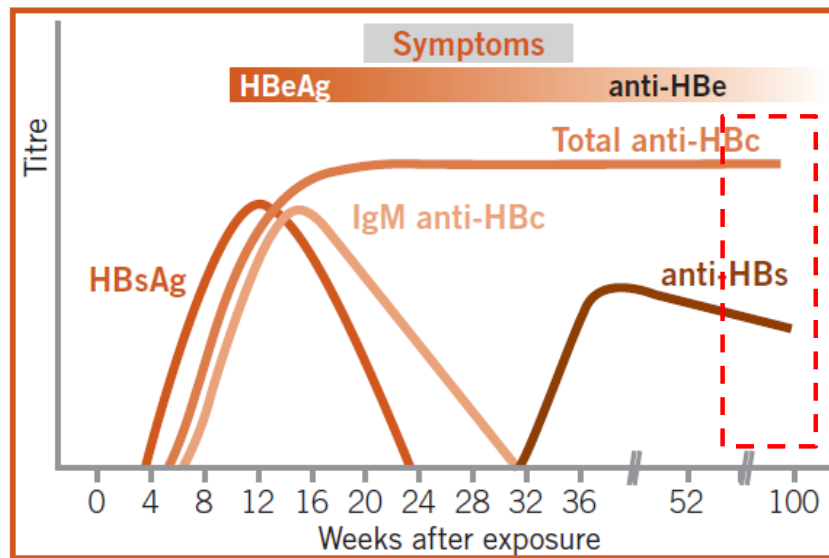
- where total anti-HBc and anti-HBs tests are positive

## 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



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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

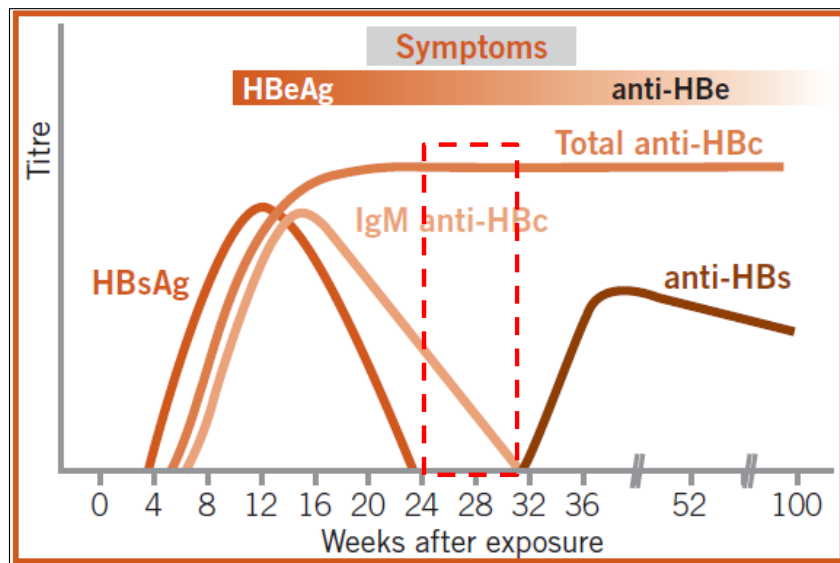
Anti-HBs only positive means “immunity due to vaccination”.

## 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



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In this figure illustrate the above slide on “recent infection, recovered, immunity achieved”.

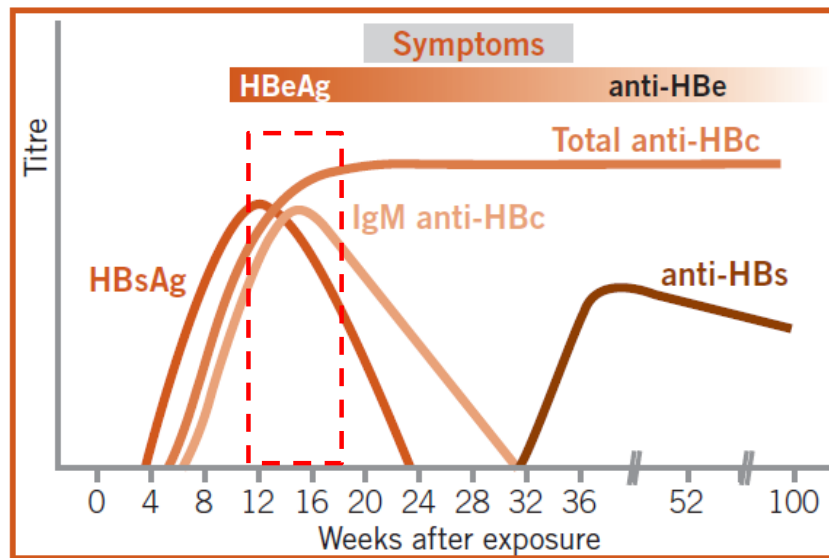
Noted the red dash box: Anti-HBs levels have dropped and disappeared.

## 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



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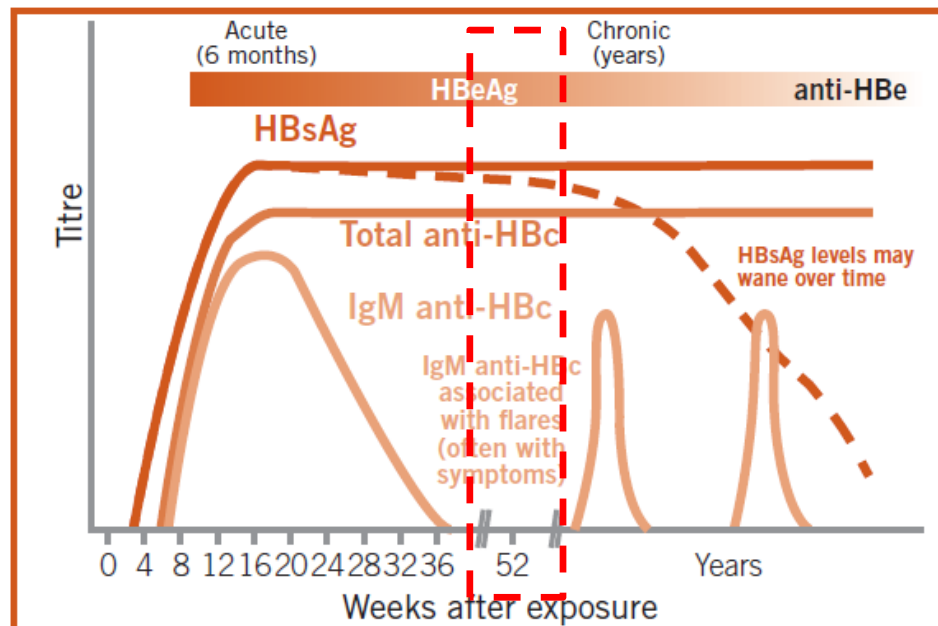
In this figure (red dash box) illustrates that “acute infection, is ongoing”, and where HBs is positive , total anti-HBc is positive, IgM anti-HBc is positive and anti-HBc is negative

## 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



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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

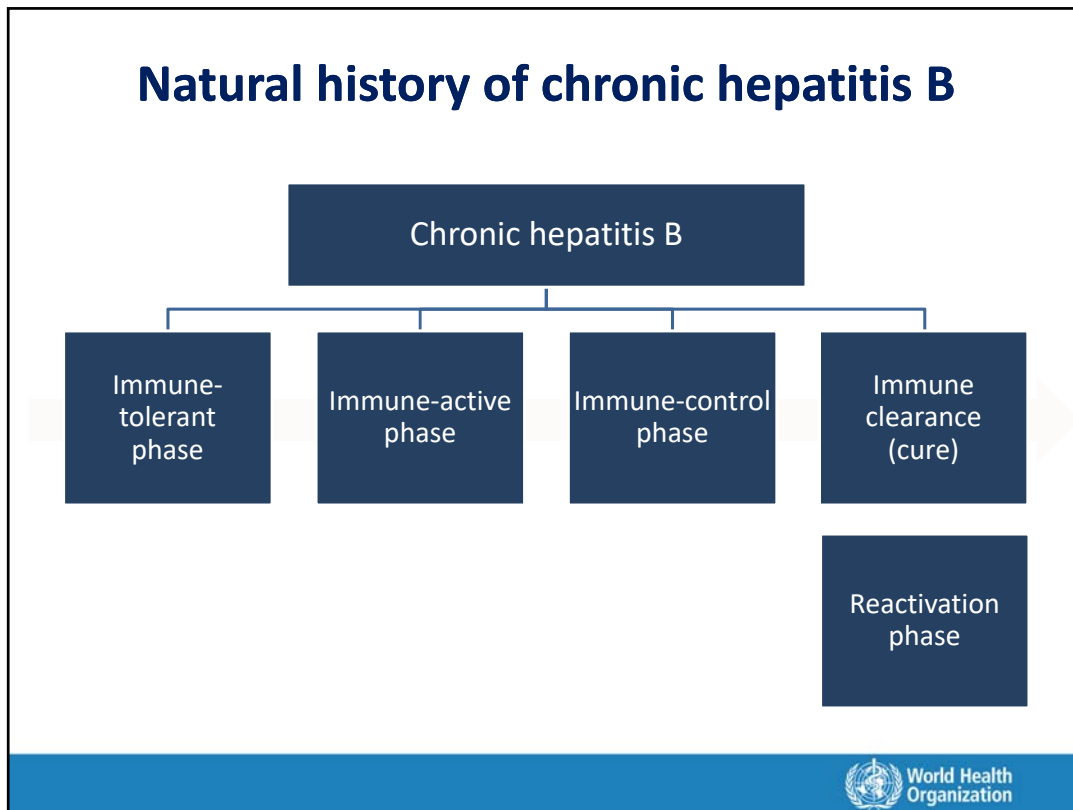
HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
-			-	Never exposed
-	+	-	+	Past natural infection, cleared, immunity achieved
-	+	-	-	Past natural infection, cleared, anti-HBs has waned over time
-	-	-	+	Immunity due to vaccination
-	+	+	+	Recent infection, recovered, immunity achieved
+	+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

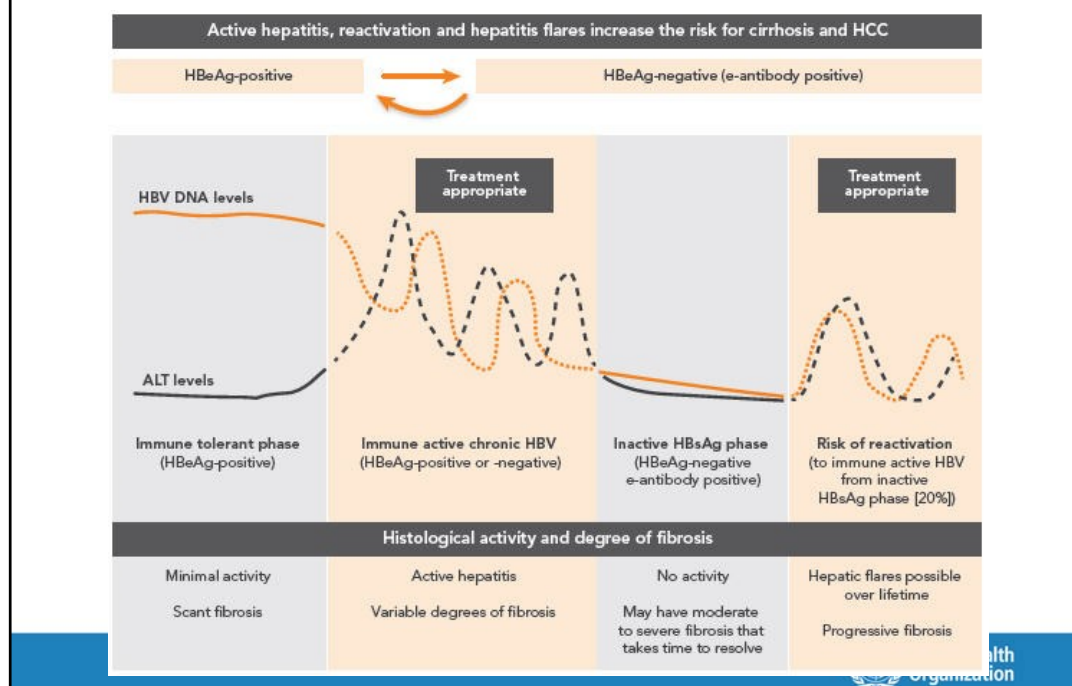




The natural history of chronic hepatitis is shown in this slide.

- There are basically 4 phases, immune-tolerant phase, immune-active phase, immune-control phase and immune clearance, cure phase.
- Another is reactivation phase, this is specific situation.

## Natural history of chronic HBV infection



This is graph of ALT level and HBV DNA level during natural history of chronic HBV infection.

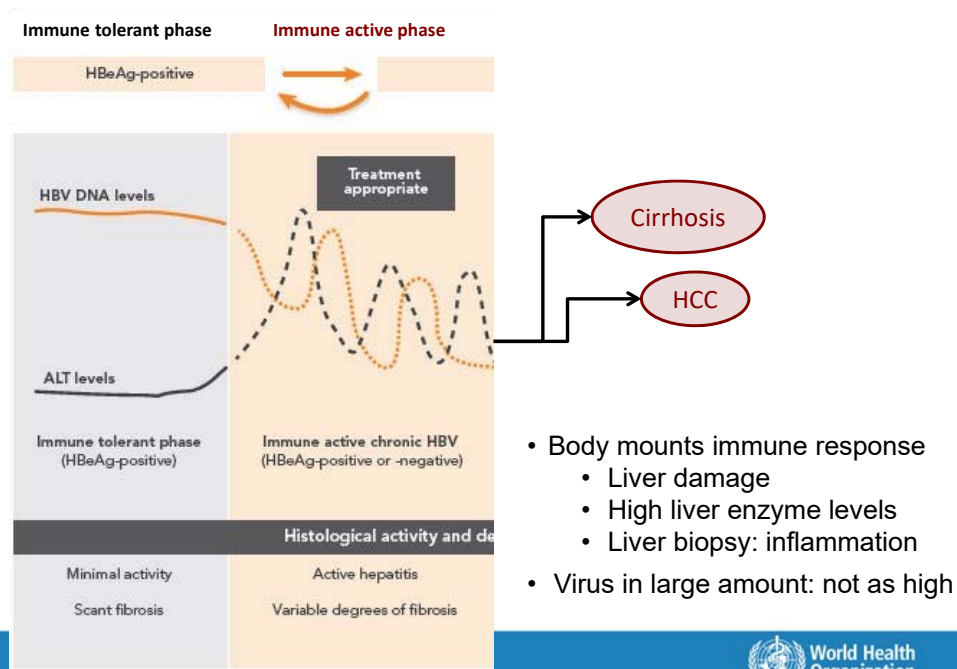
## Natural history of chronic HBV infection



- Virus in large amount
- No host immune response
  - No liver damage
  - Normal liver enzyme levels in serum
  - Normal liver biopsy

- In the immune tolerant phase, large amount of viruses in blood, but there is no host immune response.
- Therefore, there is no liver damage, and liver enzyme levels in serum is normal.
- Liver biopsy shows little inflammation.

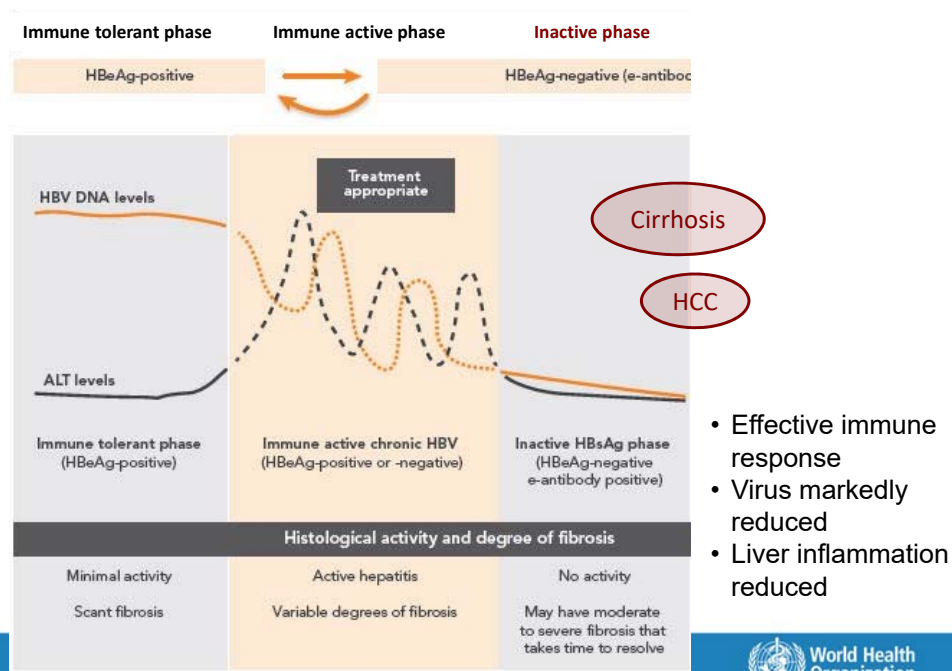
## Natural history of chronic HBV infection



In the immune active phase, the body mounts an immune response.

- The liver is damaged by host immunity.
- Liver enzyme levels are elevated and fluctuated.
- Liver biopsy shows various grades inflammation.
- But, virus levels (i.e. viral load) which is the HBV DNA, is not high compared to the immune tolerant phase and viral levels often fluctuated.
- If this phase where the viral levels remain relatively high, liver cirrhosis or HCC can develop.

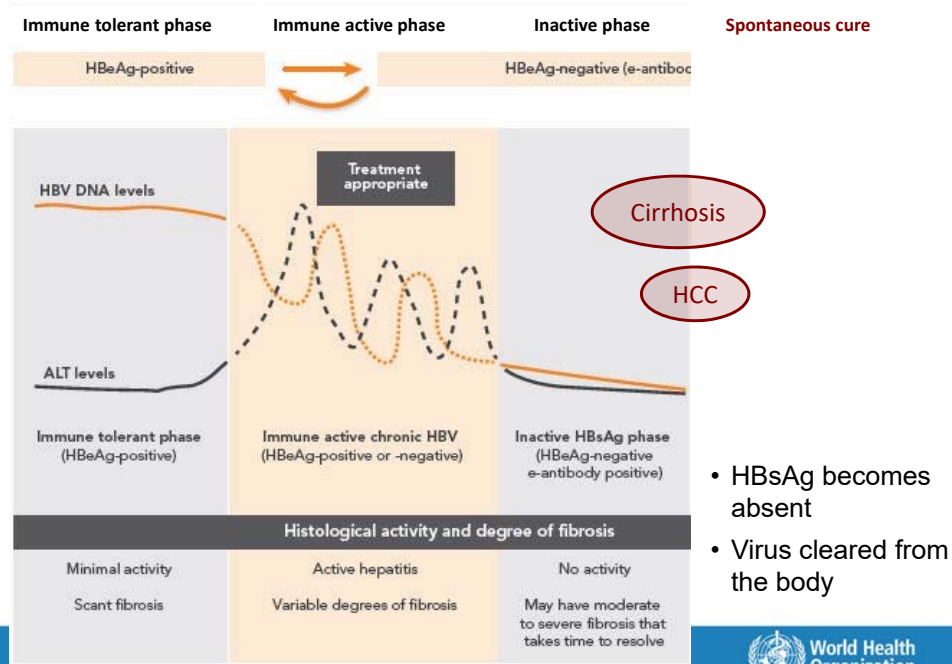
## Natural history of chronic HBV infection



In the inactive HBsAg phase, HBeAg positive becomes negative.

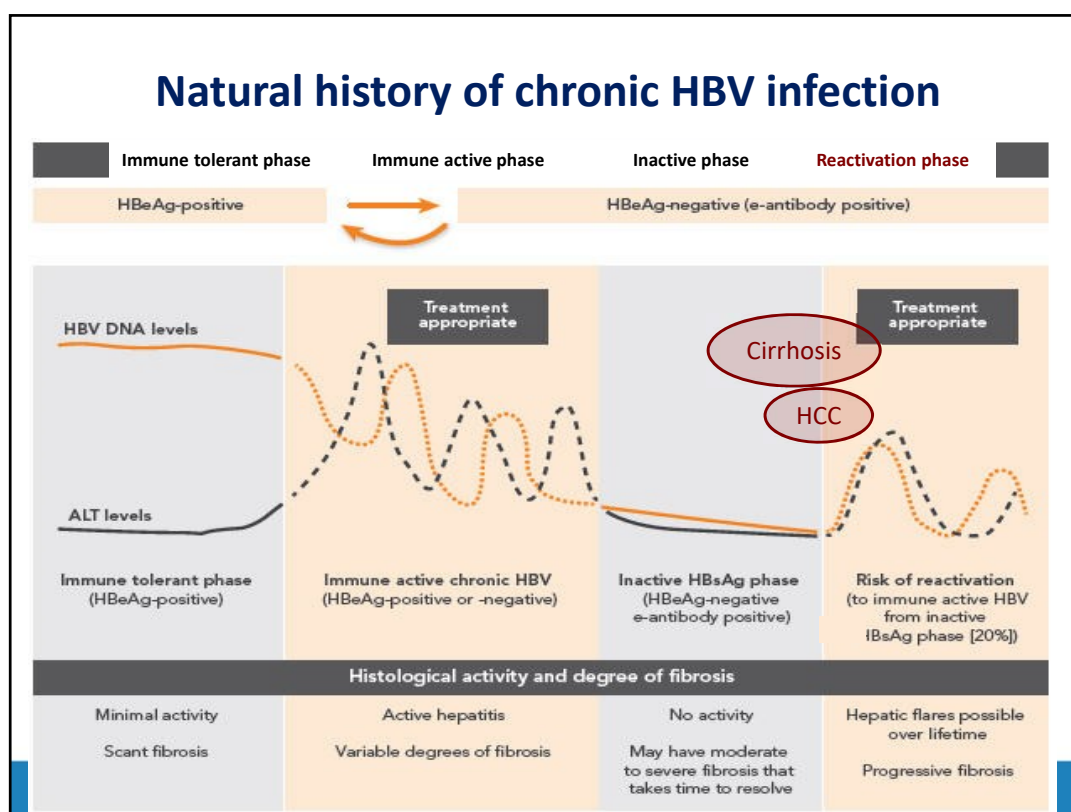
- The host immune response is effective in controlling the hepatitis B virus.
- ALT levels markedly decreases and viral load markedly reduced.
- Liver biopsy shows reduced inflammation.
- However, even in this phase, the risk for cirrhosis or HCC remain.

## Natural history of chronic HBV infection



In some case, HBsAg become absent and the HBV virus is cleared from the body.

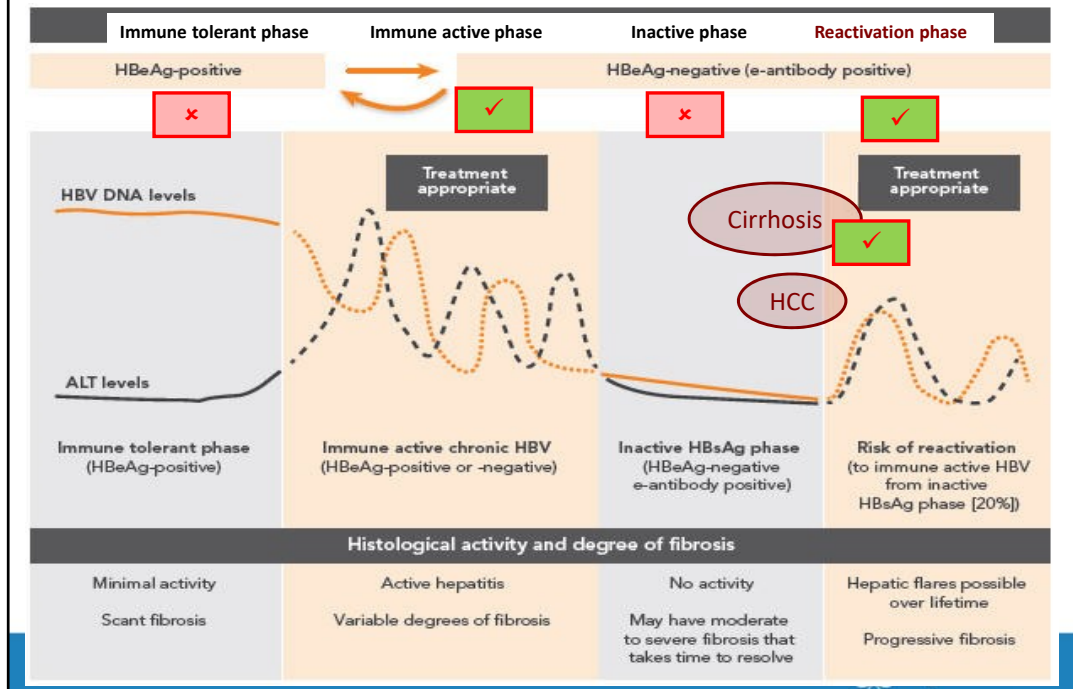
- This is immune clearance, that is, the “functional cure” phase (where HBsAg is negative, in a previously documented chronically infected individual).



In the reactivation phase, there is a sudden increase in HBV replication (where the viral load increases) in a patient with previously immune inactive stage. Reactivation can happen spontaneously, but is typically triggered by immunosuppressive therapy of cancer, autoimmune disease, or organ transplantation.

Note: among people with HBV/HCV coinfection – HBV reactivation can occur during treatment of the HCV infection, and thus may need to be provide HBV drugs during this period.

## Chronic HBV infection: Need for treatment



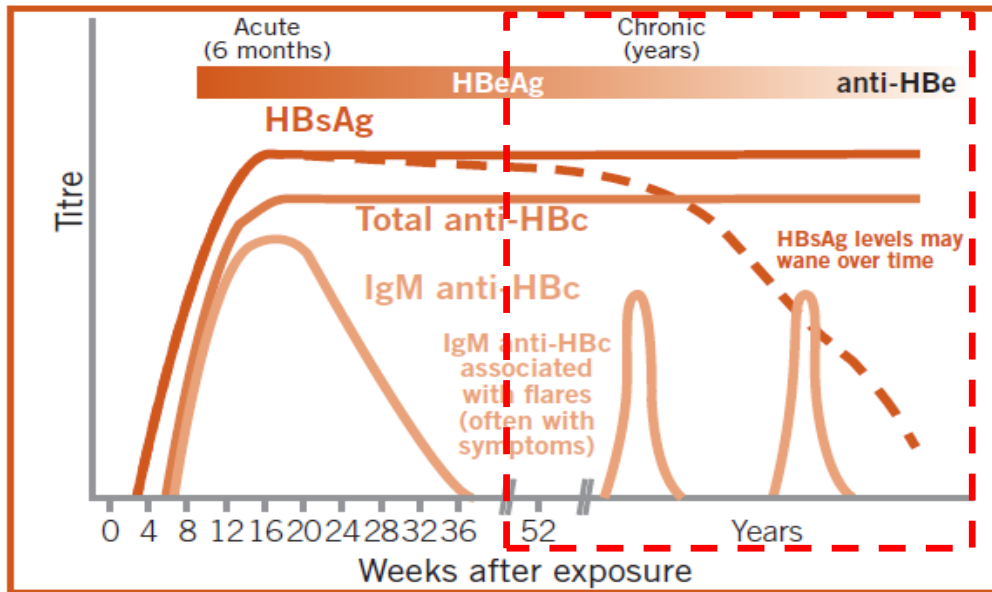
Treatment is needed for immune active phase and reactivation phase [green boxes with ticks]

Cirrhosis with any phase also need provision of treatment [green box with ticks]

On the other hand, in immune tolerant phase and inactive phase, there is no need for treatment [red boxes]



## Serological pattern of chronic HBV infection



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To review: this slide shows the serological pattern of chronic HBV infection.

- Basically HBsAg and anti-HBc continue to be positive.
- HBeAg gradually decreases and finally anti-HBe become positive (this is often called "minor seroconversion")
- In some case, IgM anti-HBc become positive with low level associated with hepatitis flare.
- HBsAg levels may wane over time as age increases (especially in elderly people).

## Approaches for detecting HBV infection

- General population testing, i.e., mass screening
- Focused or targeted testing of specific high-risk groups
- Blood donor screening
- Screening of pregnant women



Next, let's talk about approaches for detecting HBV infection.

There are four approaches for testing:

- General population testing, i.e., mass screening
- Focused or targeted testing of specific high-risk groups e.g. people living with HIV, prisoners, people who inject drugs, other at-risk groups, older people more than 40 years of age (testing by birth cohort), people who received unscreened/unsafe blood and blood products etc.
- Blood donor screening (usually compulsory for blood banks)
- Screening of pregnant women (as part of integrated antenatal services towards triple elimination of mother to child transmission of HIV, syphilis and viral hepatitis).

## Approach for testing for HBV infection

Testing approach and population	Recommendations
General population testing	In a setting with $\geq 2\%$ or $\geq 5\%$ HBV seroprevalence, all adults have access to HBV serological testing and linkage to care
Focused testing in most affected populations	<p>In all settings, serological testing for HBV antibody be offered to the following individuals</p> <ul style="list-style-type: none"> <li>● Adults and adolescents from populations most affected by HBV infection</li> <li>● High prevalence: migrants, high/intermediate prevalence, tribes</li> <li>● History of exposure</li> <li>● High-risk behaviors</li> <li>● Adults and children with a clinical suspicion of chronic viral hepatitis</li> <li>● Sexual partners, children and other family members, and close household contacts of those with HBV infection</li> <li>● Health care workers</li> </ul>

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## Approach for testing for HBV infection

Testing approach and population	Recommendations
Pregnant women screening	In a setting with $\geq 2\%$ or $\geq 5\%$ HBV seroprevalence, routine testing of pregnant women for HBV infection is recommended
Blood donors screening	In all setting, all donors have to be screened for HBV infection

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Country adaptation of the WHO guidelines for testing for hepatitis B and C is needed. Testing for target groups and people at risk should be determined

## How to test for chronic HBV infection

Topic	Recommendations
Which serological assays to use	<p>For the diagnosis of chronic HBV infection, a serological assay (in <b>either RDT or laboratory-based immunoassay format</b>) is recommended to detect <b>hepatitis B surface antigen (HBsAg)</b>.</p> <ul style="list-style-type: none"> <li>- In settings where existing laboratory testing is already available and accessible, laboratory-based immunoassays are recommended as the preferred assay format.</li> <li>- In settings where there is limited access to laboratory testing and/or in populations where access to rapid testing would facilitate linkage to care and treatment, use of RDTs is recommended.</li> </ul>
Serological testing strategies	<ul style="list-style-type: none"> <li>• In settings or populations with an <b>HBsAg seroprevalence of <math>\geq 0.4\%</math></b>, <b>a single serological assay</b> for detection of HBsAg is recommended, prior to further evaluation for HBV DNA and staging of liver disease.</li> <li>• In settings or populations with a <b>low HBsAg seroprevalence of <math>&lt; 0.4\%</math></b>, confirmation of HBsAg positivity on the same immunoassay with a neutralization step or a second different RDT assay for detection of HBsAg may be considered.</li> </ul>

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For the diagnosis of chronic HBV infection, a serological assay (in either RDT or laboratory-based immunoassay format) is recommended to detect hepatitis B surface antigen (HBsAg).

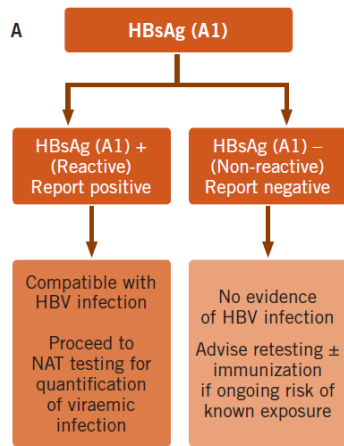
WHO outlines two strategies for HBV serological testing in setting with HBsAg seroprevalence.

- In high HBsAg seroprevalence of more than 0.4%, single serological assay.
- In low HBsAg seroprevalence of less than 0.4%, two assays with confirmation test is recommended.

## How to test for chronic HBV infection?

### (A) Single assay

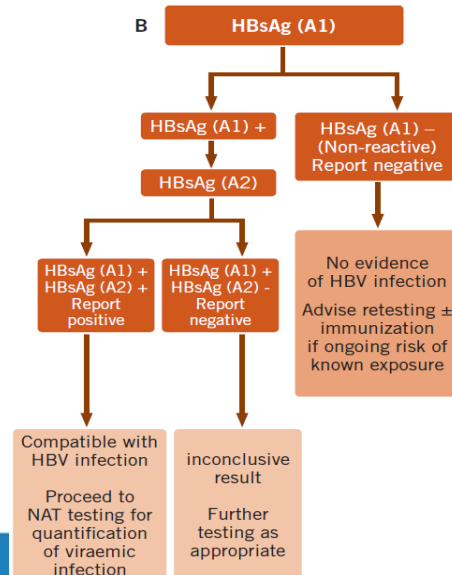
(HBs seroprevalence  $\geq 0.4\%$ )



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### (B) Two assays

(HBs seroprevalence  $< 0.4\%$ )



In high HBsAg seroprevalence, you should follow (A) single assay:

- After a positive result on the single HBsAg assay, patients can be diagnosed as having HBV infection and can proceed to NAT testing for their viral load (HBV DNA) testing

In low HBsAg seroprevalence, algorithm (B) with two assays is preferred:

- After positive on the first HBsAg assay, a second HBsAg assay is used for confirm infection status.
- When both tests are positive, patients are then diagnosed as having HBV infection and can proceed to NAT testing for their viral load (HBV DNA) testing

## Summary: Serological markers of HBV infection

- HBsAg positivity indicates current HBV infection
- If HBsAg remains positive for >6 months: chronic infection
- Presence of IgM anti-HBc implies recent (acute) infection
- Presence of anti-HBc (total) indicates
  - If HBsAg-negative: Prior exposure to HBV with clearance
  - If HBsAg-positive: Current HBV infection
- Anti-HBs indicates immunity against HBV infection, either because of prior cleared infection (anti-HBc +) or immunization (anti-HBc –)
- HBeAg, anti-HBe and HBV DNA helps in identifying the various phases in a patients with chronic HBV
- For HBV screening, 1-assay or 2-assay approach may be used, depending on disease prevalence