

Serologic and Molecular Diagnosis of HBV

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Overview

- **Definition**

- Hepatitis B virus (HBV)
- Chronic hepatitis B (CHB), Cirrhosis, Hepatocellular carcinoma (HCC)

- **Epidemiology**

- Highest prevalence (**Asia, Africa, Middle East**)
- 5% of World's population (**350-400 million**) has CHB

- **Transmission**

- Perinatal, Percutaneous, Sexual exposure, Open cuts, Sores

Overview

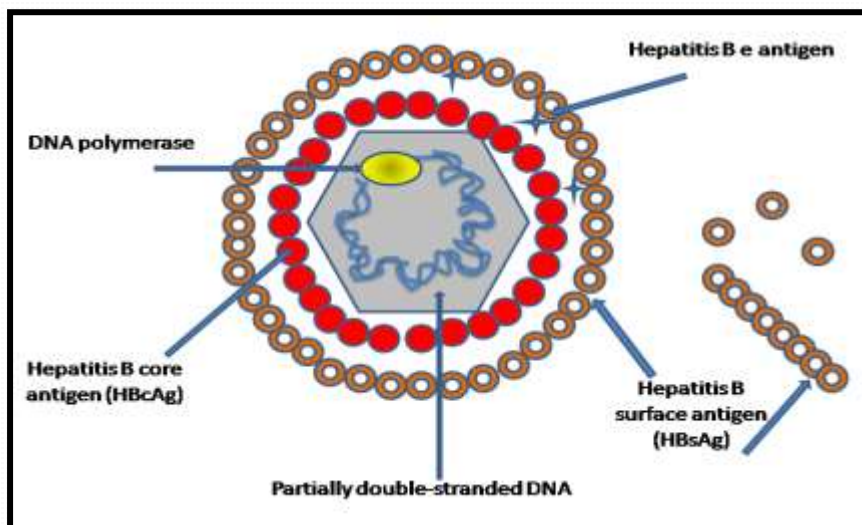
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- **Virology**
- **HBV**: Enveloped, Hepatotropic DNA viruses, Hepadnaviridae family, **3.2 kb** genome, partially double-stranded, encoding **7** proteins:
- **HBeAg, HBcAg, HBV Pol, PreS1/PreS2/HBsAg, HBx**
- **Laboratory Diagnosis**
- Enzyme immunoassay (**ELISA, RIA, CL**)
- HBV-DNA (**Real-time PCR**)

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HBV structure

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Overview

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- **Therapy**
 - PEG IFN- α , Nucleos(t)ide agents
- **Prevention**
 - Vaccine, hepatitis B immunoglobulin (**HBIG**)

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HBV serologic markers

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- **HBsAg**
 - If positive, person is infectious. **Highly sensitive** assay for HBV infection. Mutations in the **S gene** can lead to false-negative result.
- **HBsAb**
 - Immune (protective level is **>10 IU/mL**)
- **HBcAb**
 - Total HBcAb: Indicates past or active infection
 - IgM HBcAb: Acute infection, persist for 6-24 months
 - IgG HBcAb: Past/Ongoing HBV infection

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HBV serologic markers



- **HBeAg**
 - Is detectable 6-12 weeks after infection, indicates person is highly infectious, 3-4 months persistence of HBeAg: CHB
 - Selecting patients for therapy
- **Anti-HBe**
 - Prognostic for resolution of infection, less infectious spontaneous seroconversion

“No single test tells you everything”

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HBV laboratory tests

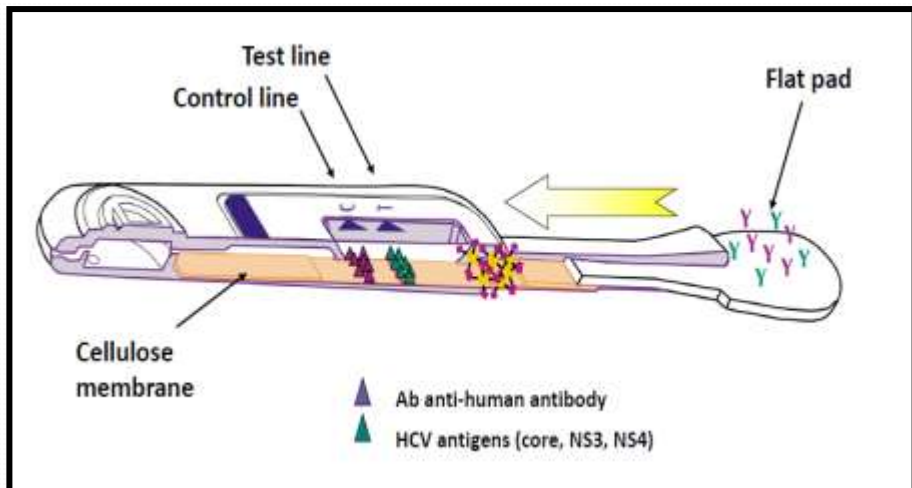


- **Serology**
 - Enzyme Immunoassays
- **Molecular**
 - HBV-DNA (qualitative, quantitative)
 - HBV genotyping
 - HBV resistance testing (Mutants)

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Rapid diagnostic test (RDT)

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Groups should be tested for HBV

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- Persons born in high or intermediate HBV endemic regions (HBsAg prevalence of $\geq 2\%$)
- Persons who have ever injected drugs*
- Men who have sex with men*
- Persons receiving immunosuppressive therapy
- Individuals with elevated ALT/AST of unknown etiology*
- Blood donors, plasma, organs, tissues, semen
- Predialysis, hemodialysis, peritoneal dialysis*
- All pregnant women
- Infants born to HBsAg+ mothers*

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Groups should be tested for HBV

- Persons with chronic liver disease, e.g., HCV*
- Persons with HIV*
- Household, needle-sharing, sexual contacts with HBsAg+ persons*
- Persons seeking evaluation or treatment for a STD*
- Health care and public safety workers
- Travelers to countries with intermediate/high HBV prevalence*
- Inmates of correctional facilities*
- Unvaccinated persons with diabetes who are aged 19-59 years
- *Indicates those who should receive HB vaccine, if seronegative.

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Primary HBV screening tests

- **HBsAg**
- **Anti-HBs Ab**
- **Anti-HBc (IgM, IgG)**

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Interpretation of HBV screening tests



Screening Test Results			Interpretation	Management	Vaccinate?
HBsAg	Anti-HBc	Anti-HBs			
+	+	-	Chronic hepatitis B	Additional testing and management needed	No
-	+	+	Post HBV infection, resolved	No further management unless immunocompromised or undergoing chemotherapy or immunosuppressive therapy	No
-	+	-	Post HBV infection, resolved or false-positive	HBV DNA testing if immunocompromised patient	Yes, if not from area of intermediate or high endemicity
-	-	+	Immune	No further testing	No
-	-	-	Uninfected and not immune	No further testing	Yes

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Isolated Anti-HBc



- About **10-30%** of Iranian healthy donors have isolated HBc Ab
 - Among HIV infected as high as 42% are isolated anti HBc
1. Previous exposure to HBV
 2. Window period
 3. False-positive result, particularly low prevalence areas
 4. False-negative HBsAg results

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Occult HBV infection markers

- HBs Ag -
- Anti-HBs -
- HBe Ag -
- Anti-HBe -
- Anti-HBc +/-
- HBV DNA +

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HBV molecular assays

- **HBV-DNA**
- Plasma/serum must be separated from cells during 6 hr and plasma can be stored at 4°C for several days or -70°C for long-term
- Quantitation of DNA: reported as **copies/mL** or **IU/mL**

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HBV-DNA level in clinical practice

- Routine monitoring of **therapy** to assess response to treatment (Every 6 months on oral agents)
- Routine monitoring of **therapy** to estimate prognosis and to evaluate need for treatment (Every 12-24 months)
- Molecular assays is **critical** to patient management

“Serology remains the cornerstone for HBV diagnosis and screening”

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Application of HBV viral load

- Diagnose cases of acute HBV
- Diagnose replicative and non-replicative chronic HBV
- Monitor patient response to antiviral therapy

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Interpretation of HBV tests



Test	Acute Hepatitis B	Immunity through Infection	Immunity through Vaccination	Immune tolerance	Immune clearance	Active chronic Infection	Inactive Carrier
HBsAg	+	-	-	+	+	+	+
HBsAb	-	+	+	-	-	-	-
HBeAg	+	-	-	+	+	+	-
HBeAb	-	+/-	-	-	-	-	+
HBcAb	+/-	+	-	+	+	+	IgG
HBcAb IgM	+	-	-	-	-	+	-
HBV DNA	+	-	-	>20,000 IU/mL	>20,000 IU/mL	>20,000 IU/mL	<2,000 IU/mL
ALT	Elevated	Normal	Normal	Normal	Elevated	Elevated	Normal

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~~viral hepatitis~~
2030

