DETECTION OF HEPATITIS B VIRUS (HBV) BY ELISA

Khder Hussein Rasul

Zahra Mohammad Maghded

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Outline

- Hepatitis B virus
- HBsAg

Objectives

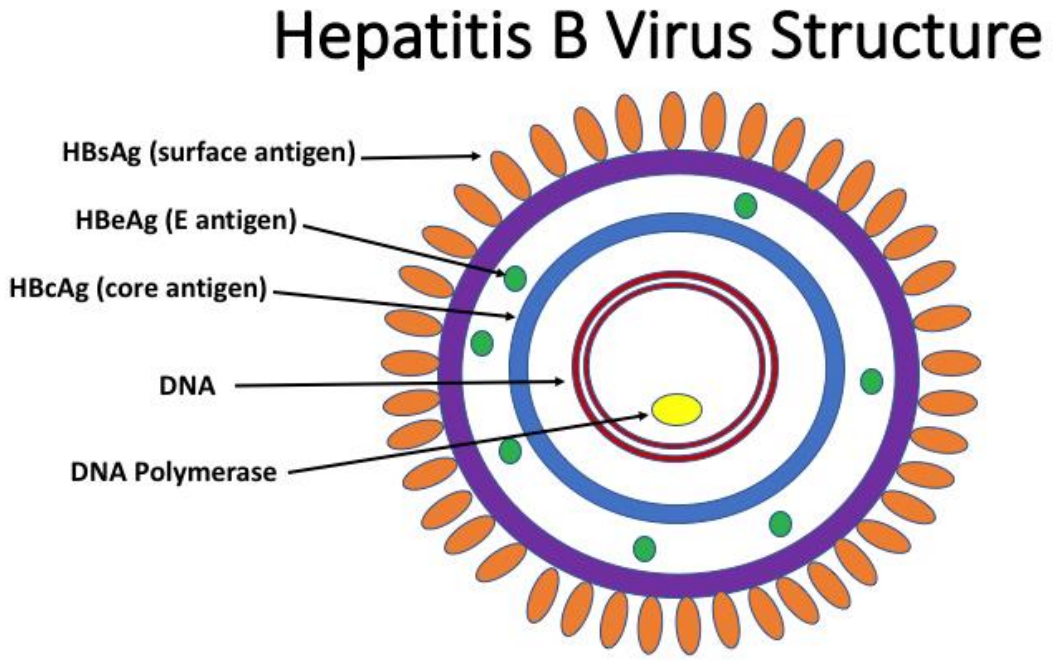
- Get knowledge about Hepatitis B virus
- Learn about HBsAg
- Get information about principle of HBsAg detection by ELISA
- Teach students about component of reagents of HBsAg ELISA kit

Hepatitis B virus

- **Hepatitis B virus** is DNA enveloped virus
- **Hepatitis B virus** is a virus that attacks the **liver** and can cause both **acute** and **chronic** diseases.
- The virus can cause chronic infection and puts people at high risk of death from **cirrhosis of the liver** or **liver cancer**.
- Hepatitis B virus is transmitted through contact with the **blood** or other **body fluids** of an infected person.
- Hepatitis B is preventable with the help of a safe and effective **vaccine** that provides protection against the virus.
- One of the crucial markers for diagnosing Hepatitis B is **HBsAg**.

HBsAg

- **HBsAg** stands for **Hepatitis B surface antigen**.
- **HBsAg** is a protein on the surface of the hepatitis B virus (HBV).
- **HBsAg** plays a vital role in the virus's ability to enter and infect liver cells.
- The presence of HBsAg in the blood is a **marker of active hepatitis B infection**.
- Testing for HBsAg is a common diagnostic tool used in the detection of hepatitis B.



Methods of HBsAg detection

1. Rapid test
2. Molecular method
3. **Enzyme-Linked Immunosorbent Assay (ELISA)**

Reagents

1. Coated Wells

One plate (9F80-01) or five plates (9F80-05) of 96 wells coated with **mouse monoclonal antibody to HBsAg.** Allow the wells to reach room temperature (18 to 30°C) before removal from the bag. Place unused wells in the sealable storage bag provided and return to 2 to 8°C.

2. Sample Diluent

One bottle containing 16 ml of green/brown buffer containing detergents and proteins of goat and bovine origin. Mix by inversion before use. Contains 0.05% ProClin® 300 preservative.

3. Negative Control

One bottle containing 2.5 ml of normal human serum. The serum is diluted in a buffer containing protein of bovine origin. Contains 0.05% Bronidox® preservative.

Reagents

4. Positive Control:

One bottle containing 2 ml of inactivated human serum. The serum is diluted in a buffer containing protein of bovine origin. Contains 0.05% Bronidox® preservative.

5. Conjugate:

One bottle containing 6 ml (9F80-01) or two bottles each containing 16 ml (9F80-05) of **horseradish-peroxidase labelled goat antibody to HBsAg** in a red buffer containing proteins of bovine and goat origin. Mix by inversion before use. Contains 0.05% ProClin® 300 preservative.

6. Substrate:

Diluent One bottle containing 35 ml of a colourless solution of tri-sodium citrate and **hydrogen peroxide**.

Reagents

7. Substrate Concentrate:

One bottle containing 35 ml of **3,3',5,5'- tetramethylbenzidine (TMB)** and stabilisers in a pink solution.

Substrate Solution

To prepare the Substrate Solution add **a volume of colourless Substrate Diluent** to **an equal volume of pink Substrate Concentrate** in either a clean glass or plastic vessel.

8. Wash Fluid:

One bottle containing 125 ml of 20 times working strength Glycine/Borate Wash Fluid. Contains 0.2% Bronidox® preservative.

Add one **volume of Wash Fluid Concentrate to 19 volumes of distilled or deionised water** to give the required volume or dilute the entire contents of one bottle of Wash Fluid to a final volume of 2500 ml.

Specimen collection, transport and storage

Specimen collection

Serum, EDTA plasma or citrate plasma samples may be used. Blood collected by **venipuncture** should be allowed to clot naturally. Ensure that the serum samples are fully clotted. Remove any visible particulate matter from the sample by centrifugation.

Specimen transport and storage

- Store samples at 2 to 8°C.
- Samples not required for assay within 72 hours should be removed from the clot or cell pellet and stored frozen (-15°C or colder).
- Avoid multiple freeze-thaw cycles. After thawing, ensure samples are thoroughly mixed before testing.

Procedure

Step 1	Prepare **Substrate Solution** and **Wash Fluid**.	
Step 2	Use only the number of wells required for the test.	
Step 3	**Add** 25 µl of **Sample Diluent** to each well.	**25 µl**
Step 4	**Add** 75 µl of **Samples** or **Controls** to the wells. To each plate add 75 µl of the Negative Control to wells A1 and B1 and 75 µl of Positive Control into well C1. Add the Controls to the designated wells afer dispensing the samples.	**75 µl**
Step 5	Cover the plate with a lid and **incubate** for 60 minutes at 37˚C ±1˚C.	**60 mins**
Step 6	**Add** 50 µl of **Conjugate** to each well.	**50 µl**
Step 7	Shake the plate using a plate shaker for 10 seconds or manually agitate by gently tapping the sides for 10 seconds.	**10 secs**
Step 8	Cover the plate with the lid and incubate for 30 minutes at 37˚C ±1˚C.	**30 mins**
Step 9	At the end of the incubation time wash the **plate** 5 times as described under **Wash Procedures**. After washing is completed invert the plate and tap out any residual Wash Fluid onto absorbent paper.	**5 washes**
Step 10	Immediately after washing the plate, **add** 100 µl of **Substrate Solution** to each well.	**100 µl**
Step 11	Cover the plate with a lid and **incubate** for 30 minutes at 37˚C ±1˚C while colour develops. A purple colour should develop in wells containing reactive samples.	**30 mins**
Step 12	**Add** 50 µl of **Stop Solution** to each well.	**50 µl**
Step 13	Within 15 minutes **read** the absorbance of each well at 450 nm using 620 nm to 690 nm as the reference wavelength if available. Blank the instrument on air (no plate in the carriage).	**$A_{450/Ref}$**

Calculation of results

Negative Control absorbance:	well 1	=	0.071, well 2	=	0.075
Mean Negative Control		=	(0.071 + 0.075)/2	=	0.073
Cut-off Value		=	0.073 + 0.05	=	0.123

Non Reactive Results

Samples giving an absorbance **less than the Cut-off Value** are considered non-reactive.

Reactive Result

Samples giving an absorbance **equal to or greater than the Cut-off Value** are considered initially reactive in the assay (see Limitations of the Procedure).

References (in APA style)

- Shih, C., et al. (2018). "Hepatitis B Virus." Trends Microbiol **26**(4): 386-387.
- Karayiannis, P. (2017). "Hepatitis B virus: virology, molecular biology, life cycle and intrahepatic spread." Hepatol Int **11**(6): 500-508.