

## Antigen ELISA Kit

96 Tests Kit

Enzyme Immunoassay for the  
Detection of Hepatitis B surface antigen  
(For In Vitro Diagnostic Use Only)  
Catalogue No. PT-HBs Antigen-96

**PISHTAZ TEB DIAGNOSTICS**

### Introduction

*Hepatitis B Virus* (HBV) infection is a world health problem. The causative virus; HBV is a member of *Hepadnaviridae* family which consists of partially double strand circular DNA. The virus has 8 different genotypes and 9 serological types which known as virus subtypes. The viral subtypes pose common *a antigen* which is subdivided into four antigenic determinants based on its amino acid types located at 122 and 166 positions of protein sequence.

The virus circulates in the blood as a round structure called Dane particle which consists of an envelope and an inner core of nucleocapsid protein, enclosing both a polymerase and the partly double-stranded circular viral DNA. Both whole intact virions and incomplete virus particles, consisting entirely of HbsAg. After HBV transmission, the early indicator of HBV infection is HBs antigen which is appear about 2-12 weeks post exposure with infected people; too earlier than any hepatic biochemical changes evidence or any obvious symptoms appear. Only sensitive diagnostic methods are capable of HBs antigen detection at this period.

In those people who recovered from HBV infection, HBs antigen is cleared from circulation 12-20 weeks after patient become symptomatic or liver specific enzymes increased. If HBs antigen detected after 6 months of post infection, the patient status known as chronic carrier. HBs antigen has a critical diagnostic role in distinguishing acute infection from chronic infection.

an hour after sample collection, refrigerate the specimen (maximum 1 week at 2-8°C) and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20°C. Avoid freeze-thaw of specimen during storage. Samples suspected to microbial contamination should not be used.

### Reagents & Specimens Preparation

1. All reagents should be allowed to reach room temperature (22-28°C) before use.
2. Working wash solution: Warm the vial at 37°C to dissolve possible crystals which formed due to concentration of solution. dilute concentrated wash solution 1:15 with distilled water before use. This solution will stable for 1 week at 2-8 °C.
3. Test steps should be done sequentially.

### Assay Procedure

1. Use required number of wells and keep the remaining with desiccants in tightly closed sealed bag. Consider first well as **Blank**, next two wells for **Negative** and another next one for **Positive controls**.
2. Add 100 µl of positive and negative controls as well as test sera into appropriate wells. At this stage nothing added into Blank well.
3. Add 25 µl of conjugate solution 1 into all wells except Blank. This turns the sample sera into pink color.
4. Seal the plate with cardboard sealer tightly. Mix gently for 15 seconds. Leave wells for 60 minutes at 37°C. (water bath/incubator).
5. **Without Wash step**, add 25 of conjugate solution 2 into all wells except Blank. Mix gently for 15 seconds. This turns the negative controls from yellow to green color and both positive control and samples color violet.

### **Materials/Equipment required but not provided with Test Kit**

1. ELISA reader with 450 and 630 nm (reference) filters.
2. Precision pipettes 25, 50 and 100 µl
3. 37°C water bath or incubator
4. 5% Sodium hypochlorite solution
5. Absorbent paper
6. Distilled water

### **General Information**

1. Do not mix kit reagents from different lot numbers. All kit components must be used only in original kit.
2. This kit is just for the detection of HBs Antigen in human serum and plasma.
3. All reagents obtained from human sources are negative for HIV Ag, HIV antibodies and HCV Ab. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.
4. Positive sera, wash solution residuals and equipments suspected to contamination by HBs Ag, should be disinfected by 5% hypochlorite solution for 30 minutes or autoclaved at 121°C for 60 minutes.

### **Storage Conditions**

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep Un-used wells in their sealed bag with desiccants. Opened kits are stable for 4 months.
3. Reagent's stability are marked on their labels. Please ensure that do not use expired date reagents.

### **Specimen Collection and Preparation**

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. Do not use diluted or pooled sera/plasmas. If testing cannot be done within

To determine positive and negative results the S/Co index is used:

$S/Co \text{ index} = \text{Sample OD} / \text{Cut-off value}$

Those with S/Co index equal or higher than 1 consider as positive and those with results less than 1 consider as negative.

### **Interpretation of Results**

1. Negative results indicate absence of or undetectable HbsAg in early stages of infection.
2. Positive results should be rechecked and those which become negative should be reported as negative. Faulty washing and sampling errors may lead to positive results.
3. In case of rechecked with positive results, confirmatory tests like Neutralization should be done.

### **Performance Characteristics**

#### **Sensitivity**

To evaluate test sensitivity, following international products are used:

1. 2nd WHO International Standard, NIBSC code 00/588
2. BBI Hepatitis B surface antigen Sensitivity panel –PHA808-

The BBI Hepatitis B surface antigen Sensitivity panel –PHA808 results are shown in table 1 (next page).

6. Cover the plate with cardboard sealer tightly and Leave wells for 30 minutes at 37°C.
7. Remove the wells content by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times (each with 300 µl of Working wash solution).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl of chromogen/ substrate solution to each well.
10. Incubate the microplate wells at room temperature and dark for 15 minutes to develop color.
11. Add 50 µl of stop solution to the wells to stop reaction.
12. Read absorbance at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available) reference filter is highly recommended.)

### **Result Calculation**

1. Measure absorbance of controls and samples at 450 nm (Use 630 nm filter as reference filter if it's available).
2. To calculate cut off value for the test, use following formulae:

$$\text{Cut-off} = \text{Negative control mean OD (450 nm)} + 0.05$$

### **Validity of the Assay**

The assay is to be considered valid if:

1. The OD 450 nm of the blank well is lower than 0.1. Higher values indicate chromogen/substrate contamination. In such a case, repeat the assay carefully and check the reagent.
2. The OD value for the negative control is lower than 0.15. Higher values indicate an incorrect washing procedure. In such a case, check the efficiency of the washing device.
3. The mean OD value of positive control is higher than the 0.8. Lower values indicate kit reagents decay. In such a case, check expiry date of the kit before repeating the assay.

**Table1. Hepatitis B surface antigen Sensitivity panel –PHA808**

Mem.ID	HBs Ag subtype	BBI ng/ml	IU/ml	PEI U/ml	S/Co Ratio Pishtazteb
1	ad	2.49	0.71	0.53	12.5
2	ad	1.17	0.31	0.20	6.5
3	ad	1.02	0.28	0.17	5.6
4	ad	0.96	0.22	0.15	4.3
5	ad	0.69	0.15	0.10	2.8
6	ad	0.50	0.12	0.08	2.5
7	ad	0.41	0.08	0.06	1.9
8	ad	0.37	0.06	0.05	1.6
9	ad	0.30	0.05	0.04	1.4
10	ad	0.23	0.02	0.02	0.9
11	ay	2.51	0.67	0.83	11.0
12	ay	1.26	0.30	0.33	5.8
13	ay	0.96	0.22	0.25	4.6
14	ay	0.77	0.19	0.19	4.1
15	ay	0.63	0.14	0.16	3.0
16	ay	0.48	0.11	0.13	2.7
17	ay	0.42	0.09	0.10	2.2
18	ay	0.33	0.06	0.08	1.5
19	ay	0.23	0.04	0.05	1.2
20	ay	0.13	0.01	0.03	0.8
21	*	0.03	0.00	0.00	0.4

The sensitivity for *ay* and *ad* subtypes is less than 0.05 IU/ml. The low titer panel is also used for kit determination.

**Table 2. HBsAg Low Titer Performance Panel (Modified) PHA106**

Member ID	Pishtazteb S/Co Ratio	HBsAg Concen. IU/ml	Abbott AxSYM S/Co	Biorad Monolisa S/Co	Ortho System3 S/Co	Behring Enzygnost S/Co
106-1	2.8	0.05	1.3	1.1	1.0	1.5
106-2	2.4	0.1	1.7	1.5	2.2	2.5
106-3	8.1	0.4	4.2	5.1	7.4	7.8
106-4	7.2	0.3	3.6	3.0	4.9	3.9
106-5	3.0	0.1	1.6	1.4	1.8	2.0
106-6	11.6	0.6	5.7	8.0	11.0	9.8
106-7	0.7	*	0.5	*	0.0	0.3
106-8	9.6	0.4	3.8	4.5	5.7	5.5
106-9	4.3	0.1	1.9	1.9	2.2	2.8
106-10	6.6	0.4	3.5	3.7	7.1	4.3
106-11	2.6	0.2	4.7	1.1	2.1	5.4
106-12	3.0	0.1	1.7	1.5	2.2	2.1
106-13	8.2	0.3	3.8	5.3	7.6	5.9
106-14	-	-	-	-	-	-
106-15	-	-	-	-	-	-

**Table 3. HBsAg Mixed Titer Performance Panel (PHA206)**

Member ID	Pishtaz teb S/Co Ratio	Abbott AxSYM S/Co	Gen.Sys S/Co	Ortho Vitros S/Co	Behring Enzygnost S/Co
206-1	0.5	0.5	0.4	0.1	0.2
206-2	>30.0	31.8	24.6	96.1	33.9
206-3	>30.0	22.4	24.6	70.0	43.9
206-4	>30.0	226	24.6	4455	43.9
206-5	>30.0	233	24.6	3999	43.9
206-6	>30.0	186	24.6	3340	43.9
206-7	29.0	21.6	24.6	56	43.9
206-8	28.0	14.9	20.5	36.7	37.3
206-9	29.0	18.7	23.6	40.7	22.2
206-10	14.0	6.3	13.2	17.7	13.4
206-11	18.0	8.1	12.1	13.4	10.6
206-12	21.0	9.8	18.2	25.5	20.3
206-13	15.0	8.2	14.2	15.1	26.2
206-14	11.0	4.8	7.6	10.4	7.9
206-15	4.0	2.6	3.6	2.4	6.6
206-16	7.7	2.8	6.6	5.3	7.1
206-17	2.7	1.6	2.2	0.7	1.7
206-18	3.4	1.7	3.4	2.0	2.9
206-19	2.8	3.0	3.3	1.6	2.6
206-20	6.8	3.6	5.3	5.9	11.8
206-21	6.0	4.6	6.7	4.9	6.8
206-22	1.8	1.2	1.6	1.3	1.6
206-23	2.8	1.4	2.5	1.8	2.4
206-24	2.7	1.3	3.7	0.9	2.2
206-25	0.8	0.7	0.4	0.1	0.4

To assess sensitivity the sero-conversion panels are also used. Following are examples of few panels used for the sensitivity evaluation.

**Table 4. Hepatitis B Seroconversion Panel (subtype ad) PHM914**

Mem ID	Days since 1st bleed	HBsAg ng/ml	Pishtaz teb S/Co	Abbott AxSYM S/Co	Biorad Monolosa S/Co	Ortho Vitros S/Co	Behring S/Co	Gen.Sys S/Co
914-01	0	<0.1	0.7	0.6	0.0	0.1	0.5	0.4
914-02	146	0.2	1.3	0.7	0.2	0.3	0.5	2.1
914-03	151	0.3	1.8	0.9	0.4	0.5	0.6	2.5
914-04	153	0.5	2.0	1.0	0.5	0.8	0.7	3.7
914-05	158	0.9	3.5	1.4	1.0	2.3	1.1	6.0
914-06	160	1.5	6.3	2.2	1.9	4.4	1.5	9.5

**Table 5. Hepatitis B Seroconversion Panel (Subtype ad) PHM909**

Mem ID	Days since 1st bleed	HBsAg ng/ml	Pishtaz teb S/Co	Abbott IMx S/Co	BioMerieux S/Co	Ortho EIA S/Co	Behring S/Co	Gen.Sys S/Co
909-01	0	<0.1	0.7	0.5	0.1	0.0	0.6	0.6
909-02	4	<0.1	0.8	0.5	0.1	0.0	0.5	0.6
909-03	7	0.1	0.9	0.6	0.1	0.0	0.6	0.8
909-04	9	0.3	1.3	0.8	0.3	0.2	0.6	2.3
909-05	14	1.1	4.7	1.5	1.0	2.1	1.8	10.3
909-06	18	>2.7	11.1	2.8	5.5	7.1	5.6	19.9
909-07	21	>2.7	20.0	5.6	13.3	16.2	11.6	23.3

**Specificity**

To evaluate test specificity, 2025 random serum/plasma samples were tested with this kit. Results showed 3 positive samples which on re-check 2 samples remained positive and one became negative.

**Table 6. The kit specificity**

Samples	Positive samples in primary test	Positive samples in test repeat	Confirmed positive test
2025	2	1	1

**Test Precision**
**Table 7. Intra-assay test results**

	Number of Repeats	Mean OD	SD	CV%
<b>Positive Sample</b>	20	2.2	0.078	3.5
<b>Positive Samples</b>	20	1.35	0.067	4.9
<b>Positive Samples</b>	20	0.2	0.0095	4.8
<b>Negative Samples</b>	20	0.055	0.0028	5.1

**Table 8. Inter-assay test results**

	Number of Repeats	Mean OD	SD	CV%
<b>Positive Sample</b>	20	2.1	0.105	5.0
<b>Positive Samples</b>	20	1.3	0.072	5.5
<b>Positive Samples</b>	20	0.2	0.014	7.0
<b>Negative Samples</b>	20	0.05	0.0042	8.4




**References**

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2. K.Kidd Ljunggren and et al . Clinical and Serological Variation Between Patients Infected with Different Hepatitis B Virus Genotypes. J Clin Microbiol 2004,42(12):5837-5841
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4. A.J.Zuckerman. Special Serological Diagnosis of Viral Hepatitis.British Medical Journal. 1979,84-86
5. D.Chen and L.A.Kaplan. Performance of a New Generation Chemiluminescence Assay for Hepatitis B Surface Antigen.Clin Chem,2006,52(8):1592-1598

## HBs Antigen ELISA Kit

### HBs Antigen ELISA Test Procedure




#### Step 1

Reagent	Control Serum	Blank	Sample
			
Control Serum (P/N)	100 $\mu$ l	None	None
Sample	None	None	100 $\mu$ l
Conjugate Solution 1	25 $\mu$ l	None	25 $\mu$ l

Cover the microplate wells with cardboard sealer tightly, Mix gently for 15 seconds and incubate them for 60 minutes at 37°C.



#### Step 2

Conjugate Solution 2	25 $\mu$ l	None	25 $\mu$ l
			

Cover the microplate wells with cardboard sealer. Mix gently for 15 seconds and incubate for 30 minutes at 37°C.






#### Step 3

Remove plate cover and discard reagents of wells. Wash the microplate wells for 5 times according to test manual.






#### Step 4

Chromogen Substrate solution	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l
			

Incubate wells for 15 minutes at room temperature and dark.



#### Step 5

Stop Solution	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l
			

Read absorbance at 450 nm (Use 630 nm filter as referen