

# HEALTH-CHEM DIAGNOSTICS, LLC

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## ONE-STEP SERUM/PLASMA TEST (HBV 5 IN 1 TEST)

### Intended Use

The HBV 5 in 1 test is an immunochromatography based one step *in vitro* test. It is designed for the qualitative determination of HbsAg antigen, Anti-HBsAg antibodies, HBeAg envelope antigen, anti-HbeAg antibodies and antiHbcAntigen core antibodies in human serum specimens within one device.

### Summaries and Explanations

#### HBsAg

The discovery of Australian antigen by Blumberg et al and its subsequent identification as the surface antigen of the Hepatitis B virus, represents a significant breakthrough in understanding the disease. Screening blood donors for the presence of the Hepatitis B virus in serum has significantly reduced the incidence of Hepatitis B in blood transfusion recipients.

The chemical structure of the Hepatitis B antigen consists of a lipid, a carbohydrate and a protein. The protein moiety of the Hepatitis B antigen includes several polypeptides, ranging from 23,000 to 97,000 KD in molecular weight. The antigenic determinant of the protein moiety of the Hepatitis B antigen determines the specific characteristics of the different serotypes of the virus and is the base of the immunoassay. The antigenic reactivity of the Hepatitis B antigen is also associated with the spherical or tubular particles on its surface. Other particles, called Dane Particles, have also been observed which have two different antigenic sites: a superficial one, identifiable as Hepatitis B surface antigen (HBsAg), an inner one, identifiable as the core. HBsAg has an antigenic heterogeneity. The principal determinant is called a (a<sub>1</sub>, a<sub>2</sub>, a<sub>3</sub>) and is common to all the different serotypes of HBsAg. Two couples of subspecific determinants have been identified: d/y and w/r. Therefore, the following combinations are possible: **adw, adr, ayw, ayr.**

The HBsAg Test consists of a chromatographic absorbent membrane strip which is immobilized with unique polyclonal specific HBsAg antibodies with a high degree of sensitivity. The antigen in sample reacts with a colored conjugate of a monoclonal specific to HBsAg, which is pre-dried on the

strip, and an antigen-antibody complex is formed when antigen is present in the sample. The mixture then moves upward and the immuno complex labeled with dye will be captured by the polyclonal antibody immobilized on the membrane, then a color band can be seen. Within 20 minutes, the test can detect levels as low as 1 ng/ml HBsAg in serum sample.

In the test procedure, serum, or plasma is added to the sample well with the aid of a dropper, and allowed to migrate through the absorbent device. The labeled antibody-dye conjugate binds to HBsAg in the serum and migrates along the chromatographic membrane through capillary action. If there is *HbsAg* present in the sample, a rose color band appears in the test window. In the absence of HBsAg, there is no formation of a rose-pink color band in the positive reaction zone. The reaction mixture continues flowing through the absorbent device past the positive reaction zone to the control zone. Unbound conjugate binds to the reagents in the control zone, producing a rose-pink color band, demonstrating that the reagents and device are functioning correctly.

#### HBsAb

HBsAb test is a sandwich immunoassay. When serum is added to the sample pad, it moves through the conjugate pad and mobilizes gold HBsAg conjugate that is coated on the conjugate pad. The mixture moves along the membrane by capillary action and reacts with HBsAg that is coated on the test region. If anti-HBsAg antibody is present, the result is the formation of a colored band in the test region. If there is no HbsAg antibody in the sample, the area will remain colorless.

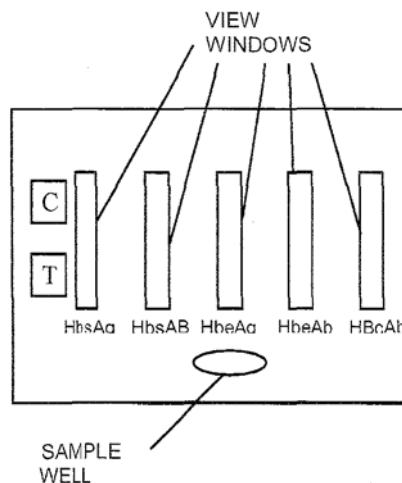
#### HBeAg

HBeAg test is a sandwich immunoassay. When serum is added to the sample pad, it moves through the conjugate pad and mobilizes gold anti-HBeAg antibody conjugate that is coated on the conjugate pad. The mixture moves along the membrane by capillary action and reacts with HBeAg that is coated on the test region. If HBeAg is

present, the result is the formation of a colored band in the test region. If there is no HBeAg in the sample, the area will remain colorless.

### HbeAb

HBeAb test is a sandwich immunoassay. When serum is added to sample pad, it moves through the conjugate pad and mobilizes gold HbeAg (recombinant) conjugate that is coated on the conjugate pad. The mixture moves along the membrane by capillary action and reacts with HBeAg (recombinant) that is coated on the test region. If anti-HBeAg antibody is present, the result is **No color line** shown in the T region. If there is no anti-HBeAg antibody in the sample, the T region will show a color band. The sample continues to move to the control area and forms a pink to purple color, indicating the test is working and the result is valid.



2. Read time: 20 minutes

### HBcAb

HBcAb test is a sandwich immunoassay. When serum is added to the sample pad, it moves through the conjugate pad and mobilizes gold HbcAg (recombinant) conjugate that is coated on the conjugate pad. The mixture moves along the membrane by capillary action and reacts with HBcAg (recombinant) that is coated on the test region. If anti-HBcAg antibody is present, the result is **No color band in T line**. If there is no anti-HBcAg antibody in the sample, the T line will show a color band. The sample continues to move to the control area and forms a pink to purple color, indicating the test is working and the result is valid.

### Materials Supplied

1. Test device.
2. Disposable dropper
3. Instructional manual

### Specimen Collection and Preparation

This test can be performed on either serum or plasma. It is recommended that fresh samples be used if possible. If this is not possible, samples should be stored in a refrigerator (2-8°C) before being analyzed. For long term storage, specimens should be frozen at -20°C.

### Assay Procedure

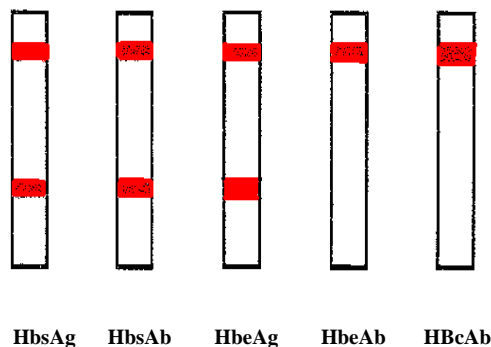
1. Using a pipette, dispense **12 - 14 drops** of specimen onto each Test line marked with "T" in the view windows. Start timing. Refer to the chart below for how the samples should look.

### Result Interpretation

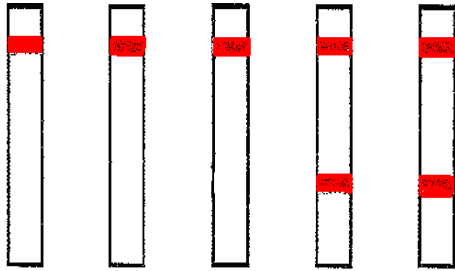
Each view window will display the results of each antigen or antibody labeled. Read the results separately.

The appearance of a pink color band in the test region (marked with a "T") will indicate whether the result is positive or negative. Illustrations of the results are as follows. Please refer to "Summaries and Explanations" on the previous page for further information.

### Positive Result:



**Negative Result:**



**HbsAg   HbsAb   HbeAg   HbeAb   HBcAb**

**Invalid Result:** If a color band does not appear in the control region “C, the test results are invalid. The sample may have been added to the wrong window, or the Test Device may have deteriorated. This specimen should be re-tested using a new Test Device.

**Precautions**

1. For In-vitro use only.
2. Do not use after expiration date.
3. Do not use reagents from different kits.
4. Store at reagents 4 -- 30 °C. Do not freeze.
5. Devices should be kept dry in the foil pouch with desiccant. Allow the strips and pouch to equilibrate to room temperature before opening the pouch to avoid condensation of moisture onto the strips.
6. Do not smoke, eat or drink where testing is conducted.
7. Do not mouth pipette. Universal precautions should be practiced. PVC gloves and proper protective eyewear and clothing should be worn. Wash hands thoroughly afterwards.
8. Infectious specimens and nonacid-containing spills should be wiped thoroughly with 5% sodium hypochlorite.
9. All waste materials should be properly disinfected before disposal. Liquid and solid wastes should be autoclaved for at least 1 hour at 121.5 °C.
10. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.

**Limitations of the Procedures**

1. Use fresh samples whenever possible. Frozen and thawed samples (especially repeatedly) contain particulate that can block the membrane. This slows the flow of reagents and can lead to high background color, making the interpretation of results difficult. (See remarks on Frozen Specimens).
2. Optimal assay performance requires strict adherence to the assay procedure described in this insert sheet. Deviations may lead to aberrant results.
3. A repeatedly positive result in this test is presumptive evidence of the presence of antibodies

to HBV in the specimen. A negative result indicated

the likely absence of detectable level to HBV in the specimen, but it does not exclude the possibility of exposure to or infection with HBV.

4. False positive and negative results might be expected with a test kit . The proportions of false results will depend on the sensitivity and specificity of the test, and on the prevalence HBV in the population to be screened.
5. Caution should be used when interpreting results of this test with pro-diluted samples.

Manufactured in the USA by:

**HEALTH-CHEM DIAGNOSTICS LLC,  
US FDA & ISO Certified Facilities  
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