

HEALTH-CHEM DIAGNOSTICS, LLC

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For the One-Step Detection of *Hepatitis B Surface Antigen (HBsAg)*

I. INTENDED USE

The HBsAg test is rapid, one-step test for the qualitative detection of Hepatitis B surface Antigen in the human serum specimens.

II. SUMMARY & EXPLANATION

The discovery of Australian antigen by Blumberg, et.a. and its subsequent identification as the surface antigen of Hepatitis B virus, represents a significant breakthrough in understanding the disease. Screening blood donors for the presence of 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 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 have also been observed, called Dane Particles, which have two different antigenic sites: a superficial one, identifiable as Hepatitis B surface antigen (HBsAg), and an inner one identifiable as the core.

HBsAg has an antigenic heterogeneity. The principal determinant is called a (a₁, a₂, a₃) 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.

III. PRINCIPLES OF THE TEST

HBsAg Test consists of a chromatographic absorbent membrane strip immobilized with a 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. 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, producing a red-color band. Within 20 minutes, the test can detect levels as low as 1-2 ng/ml HBsAg in serum sample.

IV. MATERIALS PROVIDED

1. Test Device
2. Dropper
3. Instruction manual

VI. STORAGE AND STABILITY

HBsAg test device may be stored at ambient temperature of 20 - 30°C (50-85°F) in the original unopened foil pouches. Each Test Unit contains a desiccant. The test should be used immediately once the pouch has been opened. In case the temperature of the Test Unit is considerably below room temperature and the humidity of the air is high, it is advisable to let the Test Unit reach room temperature before opening the pouch. The shelf-life of HBsAg Test Unit is 18 months from the date of manufacture. The expiration date is printed on the box.

VII. SAMPLE COLLECTION AND STORAGE

1. The HBsAg test may be performed using human serum or plasma.
2. HbsAg is thermolabil. If specimen are not immediately tested, they should be refrigerated at 2-8°C. For storage periods greater than 3 days, freezing is recommended.
3. Specimen containing precipitate may yield inconsistent test results. Such specimen must be clarified prior to assaying.

WARNINGS AND PRECAUTIONS

1. Wear disposable gloves while handling specimens. Wash hands thoroughly afterwards.
2. Wipe up spills thoroughly using an appropriate intermediate to high level disinfectant.
3. Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials, as if they were infectious, in a biohazard container.
4. Avoid splashing or aerosol formation.
5. Do not use the kit after the expiration date.
6. For *in vitro* diagnostic use only.

VIII. LIMITATIONS OF THE TEST

1. HBsAg Kit is limited to the detection of Hepatitis B virus surface antigen only.
2. Although the HBsAg Kit is very accurate in detecting HBsAg, a very low incidence of false results might occur.
3. If negative or questionable results are obtained, and Hepatitis B infection is suspected, the test should be repeated on a fresh serum specimen.
4. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after evaluation of all clinical and laboratory findings.

IX. TEST PROCEDURE

1. Remove the "Test Device" from its foil wrapper by tearing along the "splice" and place it on a clean level surface.
2. Fill the disposable dropper with the sample.
3. Hold the disposable dropper in a vertical position and apply 4 free-falling drops of sample (one by one) into the sample well of the test device.

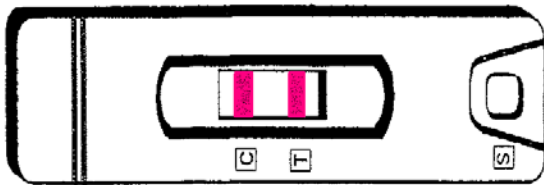
Allow each drop to soak in before adding the next one.

4. Read the results in 20 minutes.

X. INTERPRETATION

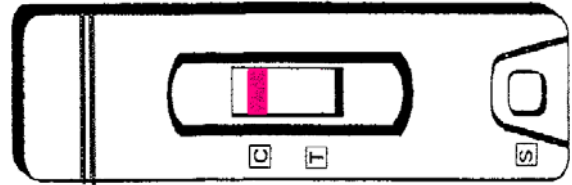
Positive Result:

If there is a rose-pink color band in the control region (marked with a "C"), and a rose-pink color band in the test region (marked with a "T"), HBsAg is present and the specimen is positive.



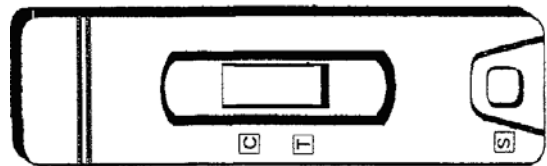
Negative Result:

The absence of a color band in the test region next to the letter "T" indicates the absence of any detectable HBsAg.



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.



XI. PERFORMANCE CHARACTERIZATION

Correlation between One-Step and ELISA

	One-Step	
	Pos.	Neg.
ELISA Pos.	149	3
ELISA Neg.	6	380

Sensitivity: *The analytical sensitivity of the Kit is 1 ng/ml for ad sub-type and 1 ng/ml for ay sub-type HBsAg in serum.*

Specificity: *The performance reactivity of the Kit for HBsAg subtypes adw, adr, ayw and ayr has been shown to be positive by utilizing standard preparations of purified antigens serially diluted in normal human serum.*

Precision: *Both inter and intra assay precision tests were run using 4 positive and 4 negative controls (1.0 and 5.0 ng/ml) with 100% correct identification each time.*

Accuracy: *A study was performed using 195 positive and 875 negative serum specimen. They were assayed by HBsAg one-step Kit and a commercially available ELISA test. The results indicated 99.4% correlation between these two tests.*

External Controls:

Like any *in vitro* device, performance of HBsAg should be checked for accuracy and batch to batch variation by using known serum pools. These sera should be used in the same way as described in the assay procedure for serum samples. It is recommended that these control

sera be used at least once with every batch or new shipment.

Internal Controls:

In addition to the external controls, the test device has built-in controls. With each testing there should always be a rose-pink color band in the control region ("C"). If the color band does not appear in the control region, the result should be considered invalid. Also, after performing the test, the result window ("T") should look clear white or uniform light pink. If the result window shows large red or purple streaks at the end of 10 minutes, the test should be considered invalid. Repeat the test using a fresh test device.

XII. REFERENCES

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Manufactured in the USA by:

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