DRG® (HBsAg) (RAP-4941)

Not for Sale in the USA

Revised 05 March 2009 **RUO**

Intended Use

HBsAg is a rapid, one-step test for the qualitative detection of *Hepatitis B surface antigen (HBsAg)* in human serum and plasma.

Summary and Explanation

The discovery of the Australian antigen by Blumberg, et. al and its subsequent identification as the surface antigen of hepatitis B virus prompted significant breakthrough in the understanding of 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 structure of the Hepatitis B antigen consists of a lipid, a carbohydrate and a protein. The protein moiety of 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 basis 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, such as the Dane Particles, which have two different antigenic sites: a superficial site, identifiable as Hepatitis B surface antigen (HBsAg), and an inner site, identifiable as the core.

HBsAg has an antigenic heterogeneity. The principal determinant is called a (there exist a1,a2,a3) and is common to all the different serotypes of HBsAg. Two pairs of subspecific determinants have also been identified: d/y and w/r. Therefore, the following combinations are possible:
adw, adr, ayw, and ayr.

Principles of the Test

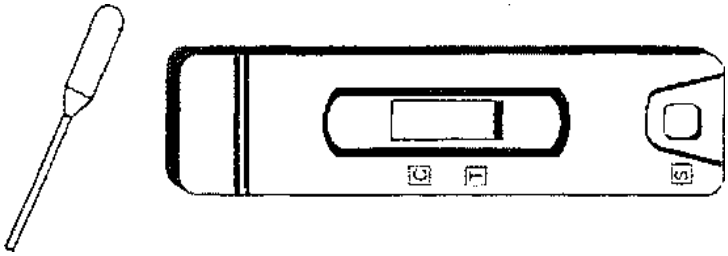
The HBsAg Test consists of a chromatographic absorbent membrane strip with a highly specific immobilized unique polyclonal HBsAg antibodies. Antigen in the serum reacts with a colored conjugate of a monoclonal specific to HBsAg , which is pre-dried onto the strip, and an antigen-antibody complex is formed when antigen is present in the sample. The mixture then moves upward and the immuno complex labeled with a dye will be captured by the polyclonal antibody immobilized on the membrane, displaying a visibly colored band. Within 20 minutes, the test can detect as low levels of HBsAg as 1 ng/ml in serum sample.

In the testing 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.

Materials Provided

1. Test Devices
2. Disposable pipettes

(packaged together in foil pouch)



PIPETTE TEST DEVICE

3. Instructions for use

Storage and Stability

HBsAg test device may be stored at ambient temperature of 20 - 30ºC (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 HBsAgTest Unit is 18 months from the date of manufacture. The expiration date is printed on the box.

Sample Collection and Storage

1. The HBsAg test may be performed using human serum or plasma.
2. HbsAg is thermolabil. If specimens are not immediately tested they should be refrigerated at 2-8^{0}C. For storage periods greater than 3 days, freezing is recommended.
3. Specimens containing precipitate may yield inconsistent test results. Such specimens 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.

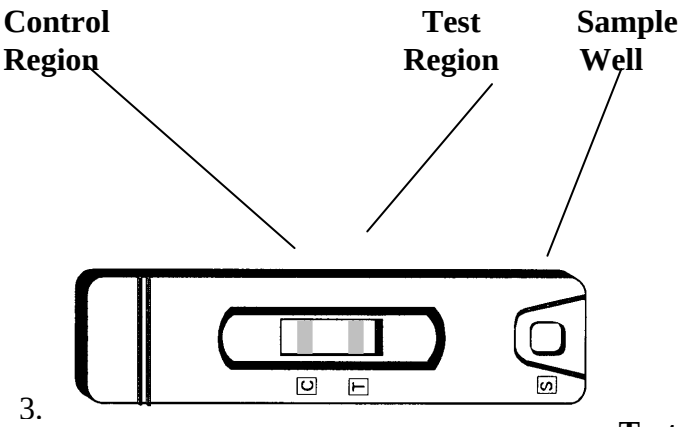
Sensitivity and specificity

Sensitivity: HBsAg-ay: 1 ng/ml
HbsAg-ad: 1 ng/ml

Specificity: 99%

Test Procedure

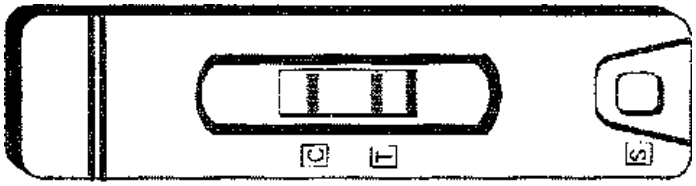
1. Bring all materials and specimens to room temperature.
2. Remove the test device from the foil pouch.



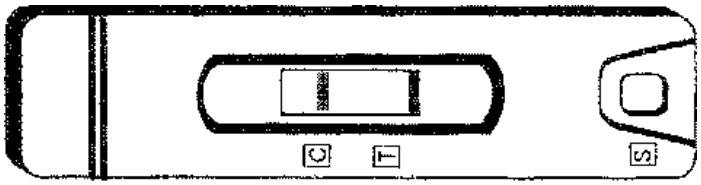
3. **Test Procedure**
4. Fill the disposable dropper with the sample.
5. Hold the disposable dropper in a vertical position and apply 2 free-falling drops of sample (one by one) into the sample well of the test device. Allow each drop to soak in before adding the next one.
6. Read the results in 10 minutes.

Interpretation

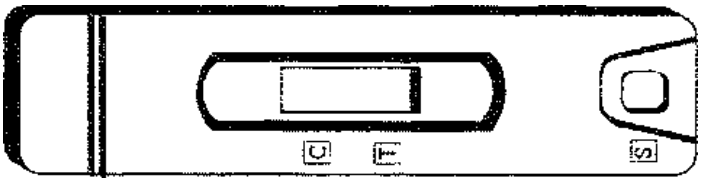
Positive Result: If there is a rose-pink color band in the control region (marked with a "C"), ***and*** a rose-pink color band in the test region (marked with a "T"), *HBsAg is* present and the specimen is positive.



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.



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 detect HBsAg, a very low incidence of false results might occure.
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.

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.

PERFORMANCE CHARACTERISTICS

1. **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.
2. **Specificity**: The performance reactivity of 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.
3. **Precision:** Both inter and intra assay precision test were run using 4 positive and 4 negative controls (1.0 and 5.0 ng/ml) with 100% correct identification each time.
4. **Accuracy:** A study was performed using 195 positive and 875 negative serum specimens. They were assayed by HBsAg one-step Kit and a commercially available ELISA test. The results indicated 99.4% correlation between these two tests.

References:

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2. Aach,R.D.,et.al.:Detection of Australia antigen by radioimmunoassay. Proc.Natl.Acad.Sci. USA,68:1056 (1971).
3. Kim,C.Y.,et.al.:Purification and biophysical characterization of Hepatitis antigen. J.Clin.Invest. 52:1176-1186 (1973).
4. Ling,C.M.,et.al.:Radioimmunoassay for Hepatitis B virus markers. Manual of clinical immunology. Rose,N. and Friedman,H.,editors.
5. Caldwell,C.W.,et.al.:Enzyme Immunoassay for Hepatitis B and its comparison to other methods. Clin.Chim.Acta. 81:305 (1977).
6. Wolters,G.,et.al.:Enzyme linked immunosorbent assay for Hepatitis B surface antigen. J.Infect.Dis. 136:311 (1977).
7. Magnius,L.O.,et.al.:New antigen-antibody system. Clinical significance in long-term carriers of Hepatitis B surface antigen. JAMA 231:356-359 (1975).
8. Peterson,D.L.,et.al.:Structure of Hepatitis B surface antigen. J.Biol.Chem. 257(17):10414-10420 (1982).