TOYO®

INSTRUCTION FOR USE
HBsAg Test, WB/S/P
HBsAg Detection in
Whole Blood / Serum / Plasma

in vitro diagnostic test

Only for professional *in vitro* diagnostic use

Product Code: THB04
Hepatitis B Virus Surface Antigen Cassette Test.

BACKGROUND INFORMATION

Hepatitis is a general term meaning inflammation of the liver and can be caused by a variety of different viruses such as hepatitis A, B, C, D and E. Since the development of jaundice is a characteristic feature of liver disease, a correct diagnosis can only be made by testing patients' sera for the presence of specific anti-viral antigens or antibodies. Of the many viral causes of human hepatitis few are of greater global importance than hepatitis B virus (HBV).Hepatitis B is a serious and common infectious disease of the liver, affecting millions of people throughout the world.
The severe pathological consequences of persistent HBV infections include the development of chronic hepatic insufficiency, cirrhosis, and hepatocellular carcinoma (HCC). In addition, HBV carriers can transmit the disease for many years. Infection occurs very often in early childhood when it is asymptomatic and often leads to the chronic carrier state. There are more than 350 million carriers of hepatitis B virus (HBV) in the world today, with addition of 4 million acute infections every year. Around one million die annually from hepatitis B-associated liver disease including hepatocellular carcinoma.
Detection of hepatitis B surface antigen (HbsAg) identifies individuals infected with the hepatitis B virus. Serum HBV DNA concentrations quantified by real-time polymerase chain reaction (PCR) correlate with disease progression and for decisions to treat and subsequent monitoring. HBsAg is typically detected by sensitive immunoassays that uses antibody to hepatitis B surface. Point of-care testing offers significant advantages which include reduction of facility costs, rapid delivery of results, early diagnosis, nurses or technicians with a minimum of training, peripheral health care level and rapid initiation of treatment.

Interpretation of the Hepatitis B Panel		
Tests	Results	Interpretation
HBsAg, anti-HBc, anti-HBs	Negative, negative, negative	Susceptible
HBsAg, anti-HBc, anti-HBs	Negative, positive, positive	Immune due to natural infection
HBsAg, anti-HBc, anti-HBs	Negative, negative, positive	Immune due to hepatitis B vaccination **
HBsAg, anti-HBc, IgM anti-HBc, anti-HBs	Positive, positive, positive, negative	Acutely infected
HBsAg, anti-HBc, IgM anti-HBc, anti-HBs	Positive, positive, negative, negative	Chronically infected
HBsAg, anti-HBc, anti-HBs	Negative, positive, negative	Four interpretations possible *

* Four Interpretations: 1. Might be recovering from acute HBV infection. 2. Might be distantly immune and test not sensitive enough to detect very low level of anti-HBs in serum. 3. Might be susceptible with a false positive anti-HBc. 4. Might be undetectable level of HBsAg present in the serum and the person is actually chronically infected.
** Antibody response (anti-HBs) can be measured quantitatively or qualitatively. A protective antibody response is reported quantitatively as 10 or more milliinternational units (≥10mIU/mL) or qualitatively as positive. Post-vaccination testing should be completed 1-2 months after the third vaccine dose for results to be meaningful.

DEFINITIONS

* Hepatitis B Surface Antigen (HBsAg): A serologic marker on the surface of HBV. It can be detected in high levels in serum during acute or chronic hepatitis. The presence of HBsAg indicates that the person is infectious. The body normally produces antibodies to HBsAg as part of the normal immune response to infection.
* Hepatitis B Surface Antibody (anti-HBs): The presence of anti-HBs is generally interpreted as indicating recovery and immunity from HBV infection. Anti-HBs also develops in a person who has been successfully vaccinated against hepatitis B.
* Total Hepatitis B Core Antibody (anti-HBc): Appears at the onset of symptoms in acute hepatitis B and persists for life. The presence of anti-HBc indicates previous or ongoing infection with hepatitis B virus (HBV) in an undefined time frame.
* IgM Antibody to Hepatits B Core Antigen (IgM anti-HBc): This antibody appears during acute or recent HBV infection and is present for about 6 months.

INTENDED USE

HBsAg Test is a rapid chromatographic immunoassay for the qualitative detection of Hepatitis B surface antigen (HBsAg) in human whole blood / serum / plasma.

REAGENTS

Anti-HBs monoclonal antibody, goat anti-mouse IgG polyclonal antibody and anti-HBs monoclonal antibody conjugated with colloidal gold particles.

METHOD

HBsAg Test uses solid-phase immunochromatographic technology for the qualitative detection of HBsAg in human whole blood / serum /plasma. The test is a two-site immunometric assay in which a combination of monoclonal and polyclonal antibodies is used to selectively detect HBsAg in samples with a high degree of sensitivity. Anti-HBs monoclonal antibody was immobilized on the test area "T" and goat anti-mouse IgG antibody was immobilized on the control area "C" of the nitrocellulose membrane. Anti-HBs monoclonal antibody conjugated with colloidal gold particles was dried on a conjugate pad. Sample is introduced from sampling pad. If there is HBsAg in the sample, HBsAg binds to the mobile anti-HBs monoclonal antibodies conjugated with colloidal gold particles. Together they move to the test area "T". Hepatitis B surface antigens bind to the immobilized anti-HBs monoclonal antibodies and as a result of this, HBsAg that have already bound to mobile anti-HBs monoclonal antibodies (conjugated with colloidal gold particles) become immobilized in the test area "T" thus creating a visible colored signal due to the accumulation of colloidal gold particles in the test area "T" (a colored test line), indicating positive test result. If there is no HBsAg in the sample then sample moves to the test area "T" together with unbound (free) anti-HBs monoclonal antibodies conjugated with colloidal gold particles. Immobilized anti-HBs monoclonal antibodies can not bind to mobilized anti-HBs monoclonal antibodies conjugated with colloidal gold particles, therefore no visible colored signal in the test area "T" (no colored test line) can be obtained, indicating negative test result. Regardless of HBsAg content of the liquid sample, mobile anti-HBs monoclonal antibodies conjugated with colloidal gold particles, bind to immobilized goat anti-mouse IgG antibodies while liquid sample is passing through the control area "C". Therefore accumulation of colloidal gold particles produces a visible colored signal in the control area "C" (a colored control line), indicating a valid test result. Colored line should be visible in the control area "C" in every case; if no visible colored line in control area "C", test result should be indicated as invalid.

PRECAUTIONS AND LIMITATIONS

1. For professional and *in vitro* diagnostic use only.
2. Do not use test kit beyond expiry date. The test device is single use. Do not reuse.
3. The test device should remain in its original sealed pouch until usage. Do not use the test if the seal is broken or the pouch is damaged.
4. Wear disposable gloves while performing the test.
5. Use a new dropper for each sample.
6. The test device should be discarded in a proper biohazard container after testing.
7. All patient samples should be handled as taking capable of transmitting disease into consideration. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of samples.
8. Do not freeze and thaw the serum, plasma samples repeatedly. Using of frozen and thawed samples should be avoided whenever possible, due to the blocking of the membrane by the debris.
9. Do not use turbid, hemolyzed samples. Turbid test samples should be centrifuged.
10. Hemolytic samples should not be used since they can lead to invalid or false results.
11. This test will indicate only the presence or absence of HBsAg in the sample, and should not be used as the only basis for the diagnosis of hepatitis viral infection.

As with all diagnostic tests, it should be kept in mind that an identification diagnosis can't be based on a single test result.Diagnosis can only be reached by an expert after the evaluation of all clinical and laboratory findings.

STORAGE

Test device should be kept away from direct sunlight, moisture, heat and radiation sources. Store at 4 - 30°C (39 - 86°F). Do not freeze. The test in the original packaging retains stable until expiry date at storage conditions. The test device should be used in maximum one hour after the foil is opened.
Kit components : Test cassettes, droppers and instructions for use.
Additional materials required but not provided : Sample collection tube, centrifuge and timer
Additional materials recommended but not provided : Micropipettes to deliver mentioned amount of sample in the test procedure, negative and positive control materials.

SAMPLE COLLECTION AND PREPARATION

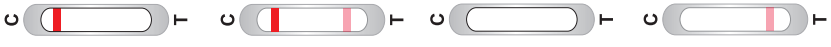
The test can be performed using whole blood, serum or plasma. To avoid hemolysis, serum or plasma should be separated from blood as soon as possible.
For Whole Blood Samples: Test should be performed immediately with whole blood samples. Otherwise, whole blood samples should be stored at 2 - 8 °C with anticoagulants (EDTA, heparin, citrate should be used) to avoid coagulation until they are being tested in a period of 2 days after collection.
For Serum Samples: Collect blood into a collection tube without anticoagulant, leave to settle for 30 minutes for blood coagulation and then centrifuge the blood. At the end of centrifuge period remaining supernatant is used as serum.(Centrifugation time & speed: 2300-2880 x g for ~ 10 min)
For Plasma Samples: Collect blood into a collection tube with anticoagulants (EDTA, heparin, citrate should be used) to avoid coagulation of blood sample and then centrifuge the blood. At the end of centrifuge period supernatant is used as plasma.(Centrifugation time & speed: 2300-2880 x g for ~ 10 min)
Do not use turbid, hemolyzed samples. If the sample cannot be tested on the day of collection, store the serum, plasma samples in a refrigerator or freezer. Do not freeze and thaw the serum, plasma samples repeatedly. Do not freeze whole blood sample. Bring the samples to room temperature before testing. Frozen samples must be completely thawed and mixed well prior to testing. Turbid test samples should be centrifuged. Using of frozen and thawed samples should be avoided whenever possible, due to the blocking of the membrane by the debris.

TEST PROCEDURE

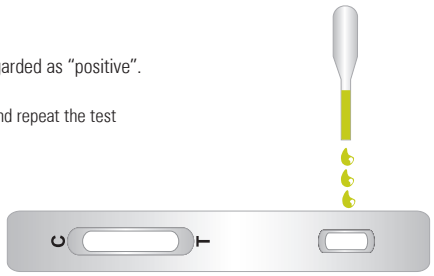
1. Bring the tests and whole blood / serum / plasma samples to room temperature, Take the test out of its pouch.
2. Draw whole blood / serum / plasma into dropper and put 3 drops (100 μl) into the sample well of the cassette. Avoid the formation of any air bubbles.
3. Depending on the HBsAg concentration in the sample, the test can react even in 5 minutes. Results should be read at 15 minutes as shown below. Do not interpret results beyond 20 minutes, results forming after 20 minutes should be regarded as invalid.

INTERPRETATION OF RESULTS

Negative: Only one colored line is visible in "C" area, indicating that hepatitis B surface antigen does not exist.
Positive: Two colored lines are visible in "C" and "T" areas, indicating that hepatitis B surface antigen exists.
Low concentration of hepatitis B surface antigen may cause a faint line in "T" area. Even such a faint line in "T" area should be regarded as "positive".
Invalid: No colored line is visible or only one colored line is visible in "T" area; test should be repeated using a new test device.
Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.



NEGATIVE POSITIVE INVALID INVALID



QUALITY CONTROL

Tests have built in procedural quality control features. When the test is complete, the user will see a colored line in the "C" area of the test on negative samples and a colored line in the "T" and "C" area on positive samples. The appearance of the control "C" line is considered as an internal procedural control. This line indicates that sufficient volume of sample was added as well as valid test result. It is recommended that a negative control and a positive control be used to verify proper test performance as an external control. Users should follow appropriate federal, state and local guidelines concerning the external quality controls.

PERFORMANCE EVALUATION

HBsAg Test can detect all subtypes of hepatitis B virus surface antigens.

Sample Status	Sample HBsAg Status	S / P Sample Type			WB Sample Type		
		Study Number	Com. Assay	Result	Study Number	Com. Assay	Result
Naturally acute or chronic infected	Positive	420	EIA	100 %	420	EIA	100 %
Blood donors	Negative	1054	EIA	100 %	1010	EIA	100 %
Clinical	Negative	250	EIA	100 %	220	EIA	100 %
Pregnant women	Negative	276	EIA	100 %	219	EIA	100 %
Rheumatoid Factor (RF)	Negative	150	EIA	100 %	150	EIA	100 %
Anti-HCV	Negative	20	EIA	100 %	10	EIA	100 %
Bilirubin	Negative	10	EIA	100 %	-	-	-
Hemoglobin	Negative	10	EIA	100 %	-	-	-
Triglycerides	Negative	10	EIA	100 %	-	-	-

Sensitivity and Specificity
For S/P samples; results of positive samples (420/420), Negative blood donors samples (1054/1054) and clinical samples (250/250) are used. Using these results; sensitivity, specificity, +predictive, - predictive values are calculated as;
Sensitivity: 100 % Specificity: 100 % + Predictive V: 100 % - Predictive V: 100 %
For WB samples; results of positive samples (420/420), Negative blood donors samples (1010/1010) and clinical samples (220/220) are used. Using these results; sensitivity, specificity, +predictive, - predictive values are calculated as;
Sensitivity: 100 % Specificity: 100 % + Predictive V: 100 % - Predictive V: 100 %

Analytical Sensitivity Cut-off: 0,26 IU/mL (0,5 ng/ml "french ng")

Seroconversion panels: 30 seroconversion panels for Türklab HBsAg Test were conducted where EIA was the reference assay. All 30 seroconversions were properly detected by the Türklab HBsAg Test which reacted positive with the appearance of a line at "C" area for the control and with another line at "T" area for the test sample. The predicate EIA assay also detected the seroconversion of all 30 panels. This evaluation indicates that Türklab HBsAg Test has demonstrated an equivalent performance in the diagnostic sensitivity compared to the CE-marked EIA assay.

Cross Reactivity: Cross reactivity has been tested with below samples, no cross reactivity was found with the HBsAg Test.
- Anti-HCV whole blood / serum / plasma samples, Anti-HCV positive control,
- Whole blood / serum / plasma samples from pregnant women.

Interferences: Following potentially interfering substances were tested with HBsAg Test: Hemoglobin, Bilirubin, Triglycerides, Rheumatoid Factor (RF). No interference was observed.

Hemolytic samples should not be used since they can lead to invalid or false results.

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Manufacturer
Consult instruction for use
Attention, see instruction for use
In vitro diagnostic medical device
For single use only
Number of test
REF Catalog number
Storage temperature
LOT Lot number
Expiry date