Rapid Response™

Hepatitis B Surface Antigen (HBsAg)
Test Strip
(Whole Blood/Serum/Plasma)

REF HBsAg-13S50 Product Insert

For professional *in vitro* diagnostic use only.

Intended Use

The Rapid Response™ Hepatitis B Surface Antigen (HBsAg) Test Strip is an *in vitro* diagnostic rapid immunochromatographic assay for the qualitative detection of Hepatitis B Surface Antigen (HBsAg) in human whole blood, serum and plasma specimens. The product may be used as an aid in the diagnosis of hepatitis B viral infection. A reactive result should be confirmed by other supplemental assay(s).

Summary

Hepatitis B is potentially life-threatening liver infection caused by the hepatitis B virus (HBV). It is a major global health problem. It can cause chronic infection and puts people at high risk of death from cirrhosis and liver cancer. About a third of the world population has been infected at one point in their lives, including 343 million who have chronic infections.[1,2,3] Many people have no symptoms during the initial infection[1]. Some develop a rapid onset of sickness with vomiting, yellowish skin, tiredness, dark urine and abdominal pain[1]. The virus is transmitted by exposure to infectious blood or body fluids[1].

The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes presented on its envelope proteins, and into eight major genotypes (A-H). The genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus. HBsAg is the surface antigen of HBV, and it indicates current hepatitis B infection. Individuals who remain HBsAg positive for at least six months are considered to be hepatitis B carriers[4]. Carriers of the virus may have chronic hepatitis B, which would be reflected by elevated serum alanine aminotransferase (ALT) levels and inflammation of the liver, if they are in the immune clearance phase of chronic infection.

It is not possible, on clinical grounds, to differentiate hepatitis B from hepatitis caused by other viral agents and, hence, laboratory confirmation of the diagnosis is essential. Laboratory diagnosis of hepatitis B infection focuses on the detection of the hepatitis B surface antigen HBsAg. WHO recommends that all blood donations be tested for hepatitis B to ensure blood safety and avoid accidental transmission to people who receive blood products.

Principle

The Rapid Response™ Hepatitis B Surface Antigen (HBsAg) Test Strip detects HBsAg through visual interpretation of color development on the internal strip. Anti-HBsAg antibodies are immobilized on the test region of the membrane.

During testing, specimen is added to the sample pad, thus stating the migration. The specimen passes the conjugate pad which contains anti-HBsAg antibodies conjugated to colloid gold particles. If there are sufficient HBsAg present in the specimen, the antigens will react and bind to the antibody-conjugate. The antigen/antibody-conjugate mixture then migrates further through the membrane and binds to antigens present on the test line, and a colored bind will form at the test region. The presence of this colored line indicates a positive result, while its absence indicates a negative result.

As liquid continues to migrate down the test strip, the control line appears. The appearance of this colored line at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

Precautions

- For professional in vitro diagnostic use only.
- Read these instructions carefully before performing the test.
- Do not use the test beyond the expiration date.
- Do not use the test if the packaging is damaged.
- Do not reuse tests.
- Apply standard biosafety precautions when handling and disposing of potentially infectious materials.
- Handle all specimens as potentially infectious.
- Wear protective clothing such as gloves, laboratory coats, eye protection when specimens are being assayed.
- The test strips and accessory should be disposed in a proper biohazard waste container after testing.
- Do not eat, drink or smoke in the area where the specimens and kits are handled.
- Avoid splashes and clean up spills immediately with appropriate disinfectant.
- The buffer containers 0.02% sodium azide as a preservative which may be toxic if ingested. When disposed of through a sink, flush with large quantities of water.
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Humidity and temperature can adversely affect results.
- Do not use any other specimen than those specified. For plasma collection, EDTA, sodium citrate, sodium heparin or potassium oxalate can be used as anticoagulant.
- Used testing materials should be discarded in accordance with local regulations.

Materials

Materials provided

- Individually packed test strips
- Droppers
- Buffer
- Product insert

Materials required but not provided

- Specimen collection equipment and container
- Centrifuge
- Timer

Storage and Stability

- The kit should be stored at 35.6-86°F (2-30°C) until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **Do not freeze the kit.**
- Protect the kit from humidity.
- Care should be taken to protect the components of the kit from contamination. Do not use the test if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

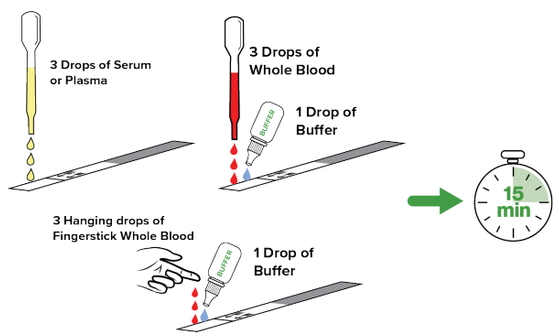
Specimen Collection and Preparation

- The Rapid Response™ Hepatitis B Surface Antigen (HBsAg) Test Strip is intended for use with human whole blood, serum, or plasma specimens only.
- Collect specimens according to safe phlebotomy procedure.
- Only clear, non-hemolyzed specimens are recommended for use with this test. Serum or plasma should be separated as soon as possible to avoid hemolysis.
- Perform testing immediately after specimen collection. Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 35.6-46.4°F (2-8°C) for up to 7 days. For long term storage, specimens should be kept below -4°F (-20°C). Whole blood collected by venipuncture should be stored at 35.6-46.4°F (2-8°C) if the test is to be run within 3 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Multiple freeze/thaw cycles should be avoided.
- If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.
- Iceteric, lipemic, hemolyzed, heat treated and contaminated sera may cause erroneous results.

Test Procedure

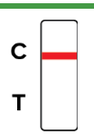
Bring the test strips, buffer and specimen to room temperature (59-86°F; 15-30°C) prior to testing.

1. Remove the test strip from its sealed pouch and use it as soon as possible. For best results, the assay should be performed within one hour.
2. Place the test strip on a clean, level surface. Label with specimen ID.
3. **For serum/plasma specimens:**
   Using the provided dropper, carefully transfer 3 drops (75 µL) of serum or plasma to the sample pad.
   For whole blood specimens:
   Using the provided dropper, carefully transfer 2 drops (50 µL) of whole blood to the sample pad, and then add 1 drop of buffer to the sample pad.
   For fingerstick whole blood specimens:
   Using the provided dropper, carefully transfer 2 hanging drops of fingerstick to the sample pad, and then add 1 drop of buffer to the sample pad.
   Avoid tapping air bubbles in the sample pad and do not add any solution to the results area.
   If the test fails to migrate across the membrane after 1 minute, add 1 drop of buffer to the sample pad.
4. Wait for the colored line(s) to appear. **Read results at 15 minutes.**

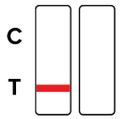


Results Interpretation

POSITIVE: Two colored lines appear. One line should always appear in the control region (C), and another line appears in the test region (T).



NEGATIVE: Only one colored line appears in the control region (C). No colored line appears in the test region (T).



INVALID: No line appears in the control region (C). Results from any test which has not produced a control line at the specified reading time must be discarded. Please review the test procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of color in the test region (T) may vary depending on the concentration of analytes present in the specimen. However, any shade of color in the test region should be considered positive. Note that this is a qualitative test only, and the concentration of analytes in the specimen cannot be determined.
2. Insufficient specimen volume, incorrect operating procedure or expired test are the most likely reasons for control line failure.

Quality Control

- An internal procedural control is included in the test. A colored line appearing in the control region (C) is considered an internal positive procedural control, confirming correct procedural technique. The control line does not control for the addition of adequate volume of specimen.
- External quality control are not supplied with this kit. It is recommended that quality controls be tested as a good laboratory practice.

Limitations

1. The Rapid Response™ Hepatitis B Surface Antigen (HBsAg) Test Strip is for professional *in vitro* diagnostic use only, and should be used for the qualitative detection of HBsAg in human whole blood, serum or plasma.
2. The Rapid Response™ Hepatitis B Surface Antigen (HBsAg) Test Strip will only indicate the presence of HBsAg in the specimen and should not be used as the sole criteria for the diagnosis of HBV infection.
3. For confirmation of test results, specimens should undergo further testing using different assays, such as rapid diagnostic tests, EIA, in accordance with validated HBsAg testing algorithm.
4. As with all diagnostic test, all results must be interpreted together with other clinical information available to the physician.
5. Results should not be used to determine the serotype of HBV infections.
6. False reactive results may arise due to damage to test components by heat or humidity, when other test kit components (e.g. droppers) are substituted between test kits.
7. Positive tests can be due to recent vaccination against HBV but this positivity is unlikely to persist beyond 14 days post-vaccination[5].
8. False non-reactive results may arise when HBsAg levels are very low or very high (hook effect), insufficient specimen volume added, or damage to test components by heat or humidity.
9. False non-reactive results may be observed in individuals who are receiving effective antiretroviral therapy.

Performance Characteristics

Genotypes and Serotypes Detection

The WHO 1st HBsAg reference panel (PEI 6100/09) was tested on the Rapid Response™ Hepatitis B Surface Antigen (HBsAg) Test Strip. Based on the study, all 15 panel members, representing subtypes A1, A2, B2, C2, D1, D2, D3, E, F2 and H, were detected with the Rapid Response™ Hepatitis B Surface Antigen (HBsAg) Test Strip.

Analytical Sensitivity

The analytical sensitivity of the Rapid Response™ Hepatitis B Surface Antigen (HBsAg) Test Strip is 2 IU/ml of HBsAg according to the PEI 6100/09.

Clinical Sensitivity, Specificity and Accuracy

The Rapid Response™ Hepatitis B Surface Antigen (HBsAg) Test Strip was evaluated with a total of 2956 specimens. The testing results were summarized in the Table below.

Rapid Response™ Hepatitis B Surface Antigen (HBsAg) Test Strip vs. EIA (*Abbott AxSYM*)

Method		ELISA		Total Results
Rapid Response™	Result	Positive	Negative	
Hepatitis B Surface Antigen	Positive	820	1	821
(HBsAg) Test Strip	Negative	1	2134	2135
Total Results		821	2135	2956

Relative Sensitivity: 99.9% (99.3%-100.0%*
Relative Specificity: >99.9% (99.7%-100.0%)*
Overall Agreement: 99.9% (99.8%-100%)*
*95% Confidence Interval

Specimen Types Consistency

50 seropositive whole blood, paired plasma and serum specimens, 50 negative whole blood, paired plasma and serum specimens were tested with the Rapid Response™ Hepatitis B Surface Antigen (HBsAg) Test Strip. Paired whole blood, plasma, and serum specimens showed consistent results with the Rapid Response™ Hepatitis B Surface Antigen (HBsAg) Test Strip.

EIA	Specimen type	No. of tested	Rapid Response™ Hepatitis B Surface Antigen (HBsAg) Test Strip	
			No. of Positive	No. of Negative
Negative	Whole blood	50	50	0
	Plasma	50	50	0
	Serum	50	50	0
Positive	Whole blood	50	0	50
	Plasma	50	0	50
	Serum	50	0	50

Precision

Intra-Assay (same kit lot)

Within-run precision has been determined by using 10 replicates of three specimens: one negative, one low positive and one high positive. One kit lot of the Rapid Response™ Hepatitis B Surface Antigen (HBsAg) Test Strip have been tested using above specimens. The specimens were correctly identified >99% of the time.

Bibliography

1. "Hepatitis B Fact sheet N°204". Whoint. July 2014. Archived from the original on 9 November 2014.
2. GBD 2015 Disease and Injury Incidence and Prevalence, Collaborators. (8 October 2016). "Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015". Lancet. 388 (10053): 1545-1602.
3. Schilsky ML (2013). "Hepatitis B "360"". Transplantation Proceedings. 45 (3): 982-985. doi:10.1002/hep.21513. PMID 17256718.
4. Rysgaard, Carolyn D; Morris, Cory S; Drees, Denny; Bebber, Tami; Davis, Scott R; Kulhavy, Jeff; Krasowski, Matthew D (2012). "Positive hepatitis B surface antigen tests due to recent vaccination: a persistent problem". BMC Clinical Pathology. 12 (1): 15. doi:10.1186/1472-6890-12-15. Retrieved 11 March 2014.

Glossary of Symbols

Consult instructions for use
Test per Kit
Do Not Reuse
Store between 35.6°F to 86°F
Use by
Catalogue #
Lot Number
For *in vitro* diagnostic use only
Manufacturer

BTNX Inc.
722 Rosebank Road,
Pickering, ON L1W 4B2
Canada
Technical Support: 1-888-339-9964