HBsAg Rapid Test Cassette (Whole Blood/Serum/Plasma) Package Insert

REF IHBSG-402 | **English**

A rapid test for the qualitative detection of Hepatitis B Surface Antigen (HBsAg) in human whole blood, serum or plasma.

For laboratory professional in vitro diagnostic use only.

【INTENDED USE】

The HBsAg Rapid Test Cassette is a rapid chromatographic immunoassay for the qualitative detection of Hepatitis B Surface Antigen in human whole blood, serum or plasma to aid in the diagnosis of HBsAg infection.

The product is intended to be used by trained laboratory personnel. For laboratory use only.

The test provides preliminary test results. Negative results will not preclude Hepatitis B virus infection and they can't be used as the sole basis for treatment or other management decision.

Not for Self-testing use. Not for near-patient use. Not for blood donor screening.

【SUMMARY】

Viral hepatitis is a systemic disease primarily involving the liver. Most cases of acute viral hepatitis are caused by Hepatitis A virus, Hepatitis B virus (HBV) or Hepatitis C virus. The complex antigen found on the surface of HBV is called HBsAg. Previous designations included the Australia or Au antigen. In a typical Hepatitis B infection, Chronic HBV infection is defined as either the presence of HBsAg in the serum for at least 6 months or the presence of HBsAg in a person who tests negative for immunoglobulin M antibodies to hepatitis B core antigen. Unlike persons who recover from acute HBV infection, persons with chronic HBV infection do not develop anti-HBs, and HBsAg typically persists for decades.[1] The presence of HBsAg in serum indicates that the patient has contracted HBV infection.[2] HBsAg has four principal subtypes: adw, ayw, adr and ayr. Because of antigenic heterogeneity of the determinant, there are 10 major serotypes of Hepatitis B virus.

The HBsAg Rapid Test Cassette is a rapid test to qualitatively detect the presence of HBsAg in whole blood, serum or plasma specimen. The test utilizes a combination of monoclonal and monoclonal antibodies to selectively detect elevated levels of HBsAg in whole blood, serum or plasma.

【PRINCIPLE】

The HBsAg Rapid Test Cassette is a qualitative, solid phase, two-site sandwich immunoassay for the detection of HBsAg in whole blood, serum or plasma. The membrane is pre-coated with anti-HBsAg antibodies on the test line region of the test. During testing, the whole blood, serum or plasma specimen reacts with the particle coated with anti-HBsAg antibodies to form a complex. The complex migrates upward on the membrane chromatographically by capillary action to react with anti-HBsAg antibodies on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

【REAGENTS】

The test contains anti-HBsAg particles and anti-HBsAg coated on the membrane.

【WARNINGS AND PRECAUTIONS】

Please read all the information in this package insert before performing the test.

•For laboratory professional use only. For *in vitro* diagnostic use only.

•Do not use after the expiration date. Do not reuse the test.

•The test should remain in the sealed pouch until use. Do not use test if the pouch is damaged.

•Do not eat, drink or smoke in the area where the specimens or kits are handled.

•Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.

•Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.

•The used test should be discarded according to local regulations.

•Humidity and temperature may adversely affect results.

•Wash hands thoroughly before and after handling.

•Any serious incident that has occurred in relation to the test shall be reported to the manufacturer and the competent authority.

•Components provided in the kit are approved for use in the HBsAg Rapid Test Cassette. Do not use any other commercial kit component.

【STORAGE AND STABILITY】

The kit can be stored at room temperature or refrigerated (2-30 °C). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. **DO NOT FREEZE**. Do not use beyond the expiration date.

Note: It is suggested to use test within one hour after removing it from the foil pouch.

【SPECIMEN COLLECTION AND PREPARATION】

- The HBsAg Rapid Test Cassette can be performed using whole blood, serum or plasma.
- **Venipuncture whole blood:**
  Collect whole blood specimen into a collection tube (with specified anticoagulant, namely EDTA K2, heparin sodium, sodium citrate or potassium oxalate) according to standard venous blood sampling process. Other anticoagulants may lead to incorrect results. Whole blood specimen can be stored at 2-8 °C for up to 2 days if it is not used immediately after being sampled. Do not freeze whole blood specimen. Before testing, gently shake the blood tube to obtain a homogeneous specimen.
- **Serum:**
  Collect whole blood specimen into a collection tube without anticoagulant according to standard venous blood sampling process. Leave to settle for 30 minutes for blood coagulation, and then spin at 1,000 to 1,200 g for 10 to 15 minutes at room temperature to obtain the serum supernatant. Don't leave samples in centrifuge after spinning.
- **Plasma:**
  Collect whole blood specimen into a collection tube (with specified anticoagulant, namely EDTA K2, heparin sodium, citrate sodium or potassium oxalate) according to standard venous blood sampling process. Gently invert the collection tube for several times and leave to settle for 30 minutes for blood coagulation, and then spin at 1,000 to 1,200 g for 10 to 15 minutes at room temperature to obtain the plasma supernatant. Don't leave samples in centrifuge after spinning.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed specimens.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8 °C for up to 3 days, for long term storage, specimens should be kept below -20 °C. Whole blood collected by venipuncture should be stored at 2-8 °C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

【MATERIALS】

Materials provided

	Kit size		1T/kit	10T/kit	20T/kit	25T/kit	40T/kit
Components	Test cassette		1	10	20	25	40
	Package insert		1	1	1	1	1
	Dropper or Capillary tube		1	10	20	25	40
	Buffer (3 mL vial or Disposable) (PBS, 0.02% Proclin 300, ≤0.02% NaN_3)	3 mL vial	1	1	1	1	2
		Disposable	1	10	20	25	40
	Sterile lancet (optional)		1	10	20	25	40
	Alcohol pad (optional)		1	10	20	25	40

Materials required but not provided

• Specimen collection containers • Centrifuge • Timer

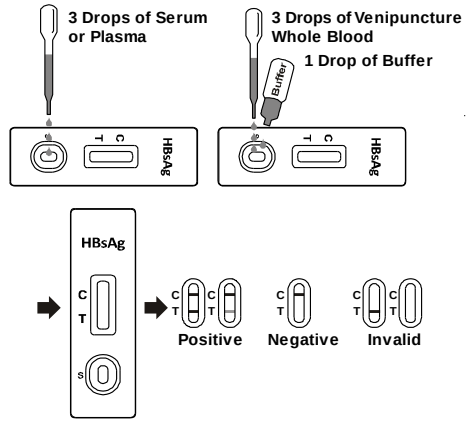
【DIRECTIONS FOR USE】

Allow the test, specimen and buffer to reach room temperature (15-30 °C) prior to testing.

1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible.
2. Place the cassette on a clean and level surface.
   For **Serum or Plasma** specimen:
   - Hold the dropper vertically and transfer **3 drops of serum or plasma** to the specimen well (S) of test cassette and start the timer. See illustration below.

   For **Venipuncture Whole Blood** specimen:
   - Hold the dropper vertically and transfer **3 drops of whole blood** to the specimen well (S) of test cassette, then **add 1 drop of buffer**, and start the timer. See illustration below.
3. Wait for the colored line(s) to appear. **Read results at 15~30 minutes.** Do not interpret the result after 30 minutes.

Note: It is suggested not to use the vial buffer beyond 6 months after opening the vial.



【INTERPRETATION OF RESULTS】

(Please refer to the illustration above)

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

***NOTE**: The intensity of the color in the test line region (T) will vary depending on the concentration of HBsAg present in the specimen. Therefore, any shade of color in the test region (T) should be considered positive.

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

INVALID: Control line fails to appear. 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. If the problem persists, discontinue using the test kit immediately and contact local distributor.

【QUALITY CONTROL】

A procedural control is included in the test. A colored line appearing in the control line region(C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

【LIMITATIONS】

1. The HBsAg Rapid Test Cassette is for professional *in vitro* diagnostic use only. The test should be used for the detection of HBsAg in human whole blood, serum or plasma specimen. Neither the quantitative value nor the rate of HBsAg concentration can be determined by this qualitative test.
2. The HBsAg Rapid Test Cassette will only indicate the presence of HBsAg in the specimen and should not be used as the sole criteria for the diagnosis of Hepatitis B viral infection.
3. Other forms of infection like seronegative infection in window period and occult hepatitis B infection could be missed by HBsAg assays.
4. When the test results and clinical symptoms are inconsistent, it should be confirmed by ELISA, CMIA or NAT.
5. The HBsAg Rapid Test Cassette cannot detect less than 1 ng/mL of HBsAg in specimens. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is suggested. A negative result at any time does not preclude the possibility of Hepatitis B infection.
6. The hematocrit of the whole blood should be between 25% and 65%.

【PERFORMANCE CHARACTERISTICS】

Sensitivity and Specificity

The HBsAg Rapid Test Cassette (Whole Blood/Serum/Plasma) was tested serum, plasma and whole blood clinical specimens and compared with CE marked CMIA test. The results show that the relative sensitivity of the HBsAg Rapid Test Cassette (Whole Blood/Serum/Plasma) is 99.87% and the relative specificity is 99.86%.

For Whole Blood/Serum/Plasma Specimen

Sample Status	Sample HBsAg Status	Comparator Method	Serum/Plasma Specimen			Whole Blood Specimen		
			Specimen Number	HBsAg Rapid Test		Specimen Number	HBsAg Rapid Test	
				Positive	Negative		Positive	Negative
HBsAg positive sample	Positive	CMIA	722	721	1	50	50	0
Blood Donation	Negative	CMIA	900	2	898	200	0	200
Clinical (hospital) sample	Negative	CMIA	1282	2	1280	30	0	30
Pregnant Woman	Negative	CMIA	215	0	215	/	/	/
Interference Substance	Negative	CMIA	140	0	140	/	/	/

Relative Sensitivity =99.87%(95%CI*:99.28%->99.99%)
Relative Specificity =99.86% (95%CI*:99. 63%-99.96%)
Overall Accuracy=99.86% (95% CI*: 99.67%-99.95%) *Confidence Intervals

Separately for Serum Specimen

Sample Status	Sample HBsAg Status	Comparator Method	Serum Specimen		
			Specimen Number	HBsAg Rapid Test	
				Positive	Negative
HBsAg positive sample	Positive	CMIA	492	492	0
Blood Donation	Negative	CMIA	800	2	798
Clinical (hospital) sample	Negative	CMIA	1062	2	1060
Pregnant Woman	Negative	CMIA	215	0	215
Interference Substance	Negative	CMIA	140	0	140

Relative Sensitivity =>99.99%(95%CI*:99.25%->99.99%)
Relative Specificity =99.82% (95%CI*:99.54%-99.95%)
Overall Accuracy=99.85% (95% CI*: 99.62%-99.96%) *Confidence Intervals

Separately for Plasma Specimen

Sample Status	Sample HBsAg Status	Comparator Method	Plasma Specimen		
			Specimen Number	HBsAg Rapid Test	
				Positive	Negative
HBsAg positive sample	Positive	CMIA	230	229	1
Blood Donation	Negative	CMIA	100	0	100
Clinical (hospital) sample	Negative	CMIA	220	0	220
Pregnant Woman	Negative	CMIA	/	/	/
Interference Substance	Negative	CMIA	/	/	/

Relative Sensitivity =99.57%(95%CI*:97.60%-99.99%)
Relative Specificity =>99.99% (95%CI*:98.85%->99.99%)
Overall Accuracy=99.82% (95% CI*: 98.99%->99.99%) *Confidence Intervals

Separately for Whole Blood Specimen

Sample Status	Sample HBsAg Status	Comparator Method	Whole Blood Specimen		
			Specimen Number	HBsAg Rapid Test	
				Positive	Negative
HBsAg positive sample	Positive	CMIA	50	50	0
Blood Donation	Negative	CMIA	200	0	200
Clinical (hospital) sample	Negative	CMIA	30	0	30
Pregnant Woman	Negative	CMIA	/	/	/
Interference Substance	Negative	CMIA	/	/	/

Relative Sensitivity =>99.99%(95%CI*:92.89%->99.99%)
Relative Specificity =>99.99% (95%CI*:98.41%->99.99%)
Overall Accuracy=>99.99% (95% CI*: 98.69%->99.99%) *Confidence Intervals

Serum vs. Plasma

Sensitivity in seropositive paired serum and plasma specimens:

A total of 100 seropositive paired serum and plasma were tested with HBsAg Rapid Test Cassette, respectively. There was a good correlation of testing results between serum and plasma with HBsAg seropositive samples.

Specimen Type	Number of specimens tested	Agreement for positive results by HBsAg Rapid Test
Serum	100	>99.9%(100/100)
Plasma	100	>99.9%(100/100)

Specificity in seropositive paired serum and plasma specimens:

A total of 220 seronegative paired serum and plasma were tested with HBsAg Rapid Test Cassette, respectively. There was a good correlation of testing results between serum and plasma with HBsAg seronegative samples.

Specimen Type	Number of specimens tested	Agreement for negative results by HBsAg Rapid Test
Serum	220	>99.9%(220/220)
Plasma	220	>99.9%(220/220)

Sero-conversion panels

30 sero-conversion panels were studied with HBsAg Rapid Test Cassette (Whole Blood/ Serum/Plasma) and compared to results from CE marked Turklab HBsAg and Biotest HBsAg tests as reference assay. HBsAg Rapid Test Cassette (Whole Blood/Serum/Plasma) has the similar detection capacity as reference assay.

Hook Effect

There is no dose hook effect with the test, when the HBsAg level is no more than 500 ng/mL.

Intra-Assay

Within-run precision has been determined by using four specimens: 0 ng/mL, 1 ng/mL, 7 ng/mL and 20 ng/mL positive specimens. The study was performed 15 replicates per day for 5 consecutive days by one operator using 1 lot of HBsAg Rapid Test, 1 lot of buffer. No difference was detected in intra lot.

Inter-Assay

Between-run precision has been determined by using four specimens: 0 ng/mL, 1 ng/mL, 7 ng/mL and 20 ng/mL positive specimens. The study was performed 15 replicates per day for 5 consecutive days in 3 different sites using 3 separate lots of HBsAg Rapid Test (one lot per site), and three operators per site. No difference was detected between days, sites, lots and operators.

Cross-reactivity

The HBsAg Rapid Test Cassette has been tested for anti-HCV, anti-HEV, anti-Syphilis, anti-EBV, CEA, AFP, PSA, CA15-3, CA19-9, CA125, anti-HAV IgM, anti-HIV, anti-RF, anti-*H.pylori*, anti-CMV IgG, anti-Rubella IgG, anti-TOXO IgG, anti-HSV 1 IgG, anti-HSV 2 IgG, Dengue NS1 and Zika NS1 positive specimens. The results showed no cross-reactivity.

Interfering Substances

The following potentially interfering substances were added to HBsAg negative and positive specimens. None of the substances at the concentration tested interfered in the assay.

Acetaminophen: 20 mg/dL
Acetylsalicylic Acid: 20 mg/dL
Ascorbic Acid: 1 g/dL
Creatin: 200 mg/dL
Bilirubin: 0.5 g/dL
Cocaine: 20 mg/dL
Caffeine: 20 mg/dL
Gentisic Acid: 20 mg/dL
Albumin: 2 g/dL
Hemoglobin: 2000 mg/dL
Oxalic Acid: 60 mg/dL
Methadone: 20 mg/dL

【BIBLIOGRAPHY】

1. Colin W. Shepard, Edgar P. Simard, Lyn Finelli, Anthony E. Fiore, Beth P. Bell, Hepatitis B Virus Infection: Epidemiology and Vaccination, Epidemiologic Reviews, Volume 28, Issue 1, August 2006, Pages 112–125.
2. Ravi Kaul,Chapter 9.17 - Hepatitis, Editor(s): David Wild, The Immunoassay Handbook(Fourth Edition),Elsevier,2013,Pages 901-911.

Index of Symbols

	Consult instructions for use or consult electronic instructions for use		Contains sufficient for <n> tests	30°C 2°C	Temperature limit
IVD	*In vitro* diagnostic medical device	LOT	Batch code	REF	Catalogue number
EC REP	Authorized representative in the European Community/European Union		Use-by date		Do not re-use
	Do not use if package is damaged and consult instructions for use		Manufacturer		Caution

Hangzhou AllTest Biotech Co.,Ltd.
#550,Yinhai Street
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Hangzhou, 310018 P.R. China
Web: www.alltests.com.cn Email: info@alltests.com.cn

CE 2934

EC REP
MedNet EC-REP GmbH
Borkstrasse 10,
48163 Muenster,
Germany

Statement: Information about manufacturer of lancet and alcohol pad is placed on the packaging.

Number: 146262003
Revision date: 2023-06-29