OnSite® HBsAg Combo Rapid Test

REF **R0042C** CE 2265

Instructions for Use

INTENDED USE

The *OnSite* HBsAg Combo Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum, plasma or whole blood. It is intended to be used by healthcare professionals as an aid in the diagnosis of infection with hepatitis B virus (HBV).

Any use or interpretation of this preliminary test result must also rely on other clinical findings and the professional judgment of health care providers. Alternative test method(s) should be used to confirm the test result obtained by this device.

SUMMARY AND EXPLANATION OF THE TEST

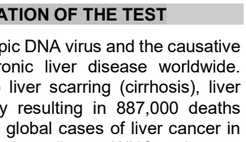
Hepatitis B virus (HBV) is a hepatotropic DNA virus and the causative agent of significant acute and chronic liver disease worldwide. Chronic HBV infections can lead to liver scarring (cirrhosis), liver failure, and liver cancer, collectively resulting in 887,000 deaths worldwide in 2015[1,2]. Of the 770,000 global cases of liver cancer in 2012, 56% were attributed to HBV[3]. According to WHO estimates, approximately 257 million people worldwide were afflicted with chronic hepatitis B infection in 2015, with only an estimated 10.5% of infected individuals globally (27 million people) aware of infection[4]. HBV carrier rates can vary from as high as 8% in high endemic areas, such as Southeast Asia and Sub-Saharan Africa, to less than 2% in low endemic areas such as the United States and northern Europe[5].

Most healthy adult individuals who are exposed to HBV clear the infection, producing neutralizing antibodies (anti-HBs) that confer lifelong immunity[6]. However, infants, children, and immunocompromised individuals exposed to HBV mostly develop chronic infections that are potential sources of transmission to others[7,8]. Diagnosis of hepatitis B infection is particularly critical as a safe and effective vaccine that confers 98-100% protection against the virus is readily available[4].

A number of well-defined antigens and antibody serological markers are available for diagnosis of HBV infection, including HBV surface antigen (HBsAg), antibodies against HBsAg (anti-HBs), and antibodies against HBV core antigen (anti-HBc)[9]. HBsAg is one of the first viral markers of infection, detectable as early as 1-2 weeks post-infection[10]. HBsAg is often detectable for the duration of clinical symptoms and is cleared upon resolution of infection. The serological persistence of HBsAg is a hallmark of chronic HBV infection, while the presence of anti-HBs is indicative of a previously resolved HBV infection or vaccination[10,11]. Thus, HBsAg can be used as a marker to monitor acute and chronic infections and any HBsAg-positive individuals should be considered potentially infectious.

The *OnSite* HBsAg Combo Rapid Test detects HBsAg in human serum, plasma or whole blood in 15-20 minutes, and can be performed by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE



The *OnSite* HBsAg Combo Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a colored conjugate pad containing murine anti-HBsAg antibodies conjugated with colloidal gold (HBsAg Ab conjugates) and a control antibody conjugated with colloidal gold; and 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line). The test line is pre-coated with a different, unconjugated murine anti-HBsAg antibody, and the control line is pre-coated with a control antibody.

When an adequate volume of specimen is dispensed into the sample well of the cassette, it migrates by capillary action across the strip. HBsAg, if present in the specimen, will migrate through the conjugate pad and bind to the HBsAg Ab conjugates. The immunocomplex is then captured on the membrane by the pre-coated HBsAg antibody, forming a colored T line that indicates a HBsAg positive or reactive test result. Lack of color development on the test line indicates a negative or non-reactive result for HBsAg.

The test contains an internal control (C line) which should exhibit a colored line by capture of the control immunocomplex by the control antibodies, regardless of color development on the T line. If the C line does not develop, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. Plastic droppers
3. Sample diluent (REF SB-R0042-CE, 5 mL/bottle of Tris-buffered solution with preservatives containing 0.095% sodium azide)
4. Instructions for Use

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer

WARNINGS AND PRECAUTIONS

For *in Vitro* diagnostic use

1. Read these Instructions for Use completely before performing the test. Failure to follow the instructions could lead to inaccurate test results.
2. Do not open the sealed pouch unless ready to conduct the assay.
3. Do not use expired devices or components.
4. Test only one specimen per device. Do not combine specimens.
5. Bring all reagents to room temperature (15-30°C) before use.
6. Do not use components from any other test kit/lot as substitutes for components in this kit/lot.
7. Do not use hemolyzed blood specimens for testing.
8. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
9. Users of this test should follow US CDC Universal Precautions for prevention of transmission of HIV, HBV and other bloodborne pathogens: https://www.cdc.gov/niosh/topics/bbp/universal.html
10. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
11. Dispose of all specimens and materials used to perform the test as biohazardous waste.
12. Handle external controls in the same manner as patient specimens.
13. Test results should be read 15-20 minutes after a specimen is applied to the sample well of the device. Reading the test result after 20 minutes should be considered invalid and must be repeated.
14. Do not perform the test in a room with strong air flow, i.e. electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All kit components are ready to use as supplied. Store unused test devices unopened at 2-30°C. If stored at 2-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze or expose the kit to temperatures above 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

Plasma/Serum

Step 1: Collect blood specimen by venipuncture into collection tube containing EDTA, citrate or heparin for plasma, or collection tube containing no anticoagulants for serum.

Step 2: To prepare plasma specimen, centrifuge collected specimen and carefully withdraw the plasma into a new pre-labeled tube.

Step 3: To prepare serum specimen, allow blood to clot, centrifuge collected specimen and carefully withdraw the serum into a new pre-labeled tube.

Test specimens immediately after collection or store refrigerated at 2-8°C for up to 5 days after collection. The specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Do not test specimens demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

Whole Blood

Step 1: Collect blood specimen by venipuncture into a collection tube containing EDTA, citrate or heparin. Do not use hemolyzed blood for testing.

Test whole blood specimens immediately after collection or store refrigerated at 2-8°C for up to 24 hours after collection. Do not freeze specimens.

ASSAY PROCEDURE

Step 1: Ensure that specimen and test components are equilibrated to room temperature. If frozen, mix the specimen well after thawing, prior to performing the assay.

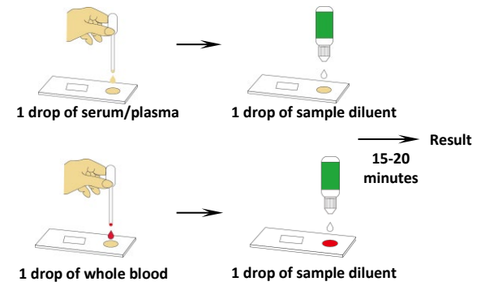
Step 2: When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.

Step 3: Label the device with the specimen ID number.

Step 4: Fill the plastic dropper with the specimen.

Holding the plastic dropper vertically, dispense 1 drop (about ~45 µL) of serum/plasma or 1 drop (about ~55 µL) of whole blood into the center of the sample well making sure that there are no air bubbles.

Immediately, add 1 drop (about ~55 µL) of sample diluent to the sample well with the bottle positioned vertically.



Step 5: Set up timer.

Step 6: Read results at 15-20 minutes. Positive results may be visible as soon as 1 minute. Negative results must be confirmed at the end of the 20 minutes. ***Any results interpreted outside 15-20 minute window should be considered invalid and must be repeated. Discard used device after interpreting the results following local laws governing the disposal of device.***

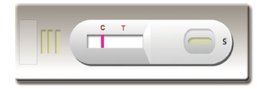
QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line that develops whether the specimen is positive or negative. If the C line does not develop, review the entire procedure and repeat the test with a new device.

2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to ensure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of test kits is used.
   d. The temperature used during storage of the kit falls outside of 2-30°C.
   e. The temperature of the test area falls outside of 15-30°C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE OR NON-REACTIVE RESULT:** If only the C line develops, there is no detectable HBsAg in the specimen. The result is HBsAg negative or non-reactive.



2. **POSITIVE OR REACTIVE RESULT:** If both the C and T lines develop, the specimen contains detectable HBsAg. The result is positive or reactive.

Specimens producing very faint test lines (indeterminate) should be re-tested with another two devices or tested with alternative method. Samples with reactive results should be confirmed with alternative testing method(s) and clinical findings before a diagnosis is made.

3. **INVALID:** If no C line develops, the assay is invalid regardless of color development on the T line as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. **Diagnostic Sensitivity**

External evaluations at three different clinical sites were performed with the *OnSite* HBsAg Combo Rapid Test and a commercial CE-marked rapid test. The data is summarized below:

Samples	*OnSite* HBsAg Combo		CE-marked Rapid Test		Relative Sensitivity
	Pos	Neg	Pos	Neg	
HBsAg Positive	486	0	486	0	100% (95% CI: 99.2-100%)

2. **Analytical Sensitivity**

38 commercially available seroconversion panels were tested with the *OnSite* HBsAg Combo Rapid Test and a commercial CE-marked rapid test. The data is summarized below.

Panels	No. of Samples	*OnSite* HBsAg Combo		CE-marked Rapid Test	
		Pos	Neg	Pos	Neg
Seroconversion	445	111	334	107	338

The OnSite HBsAg Combo Rapid Test detected 4 more HBsAg positive panel members than the reference CE-marked rapid test.

3. **Diagnostic Sensitivity – HBsAg Subtypes**

The following subtyped specimens were tested at a clinical site and detected as positive by the *Onsite* HBsAg Combo Rapid Test. The data is summarized below.

HBsAg Subtype	Samples Positive/ Samples Tested	HBsAg Subtype	Samples Positive/ Samples Tested
adw2	2/2	ayw2	1/1
ayw4	1/1	adw4	1/1
ayw1	2/2	adw	3/3
ayw3	1/1	ay	3/3
adr	4/4		

4. Analytical Sensivitiy

The limit of detection for the *OnSite* HBsAg Combo Rapid Test (defined as the concentration that yields a 95% positive detection rate) is 0.5 French ng/mL, determined using the 3rd WHO International Standard for HBsAg (NIBSC 12/226)[12].

Concentration	Samples	*OnSite* HBsAg Combo		
		Lot 1	Lot 2	Lot 3
1.0 French ng/ml	25 different matrices	100%	100%	100%
0.75 French ng/ml		100%	100%	100%
0.5 French ng/ml		96%	96%	100%
0.25 French ng/ml		36%	44%	68%
0.1 French ng/ml		0%	0%	0%

5. Diagnostic Specificity

External evaluations at three different clinical sites were performed with the *OnSite* HBsAg Combo Rapid Test and a commercial CE-marked rapid test. The data is summarized below.

Samples	*OnSite* HBsAg Combo		CE-marked Rapid Test		Relative Specificity
	Pos	Neg	Pos	Neg	
Blood donations	1*	1010	0	1011	99.9% (95% CI: 99.4-100%)
Clinical Specimens	0	701	0	701	100% (95% CI: 99.5-100%)
Pregnant Women**	0	204	0	204	100% (95% CI: 98.2-100%)
Potentially Interfering Specimens^	0	123	0	123	100% (95% CI: 97.0-100%)

*: The discordant specimen was confirmed as a true negative by CE-marked ELISA
**: Includes 20 multiparous pregnancy samples
^: Naturally-occurring specimens high in glucose, triglycerides, bilirubin, creatinine, hemoglobin, or cholesterol. In addition, no interference was observed when the following common interfering substances were spiked (at high concentrations above physiological levels) into positive and negative specimens during in-house testing: acetaminophen, aspirin, caffeine, ethanol, Fluconazole (antifungal medication), Quinine (antimalarial medication), Ethambutol (tuberculosis medication), heparin, EDTA, sodium citrate, albumin, triglycerides, glucose, HIV medication, bilirubin, creatinine, and hemoglobin.

6. Analytical Specificity – Cross-reactivity

Specimens from the following disease states or conditions were tested with *OnSite* HBsAg Combo Rapid Test. No cross-reactivity was observed.

Disease/Condition	Samples Tested	Disease/Condition	Samples Tested
HCV	10	Influenza B	2
HIV	10	TBE	3
HBc	3	HTLV-1	5
HAV	3	HTLV-2	5
Syphilis	4	Malaria	5
Toxoplasma	4	Chagas	3
HSV1	3	Influenza vaccine (IgA)	3
HSV2	3	CRP	4
E. coli	7	dsDNA	4
CMV	3	Multiparous pregnancy	2
EBV	3	RF	12
VZV	3	ANA	4
Measles	5	HAMA	2
Rubella	5	SLE	2
Influenza A	4	Yellow Fever (vaccine)	3

7. Equivalency Studies

Same day "fresh" samples (≤1 day after collection) were tested at an external clinical site with the *OnSite* HBsAg Combo Rapid Test and a CE-marked rapid test.

Sample	Matrix	No.	*OnSite* HBsAg Combo		CE-marked Rapid Test	
			Pos	Neg	Pos	Neg
HBsAg Positive	Serum	27	27	0	27	0
	Plasma (EDTA)	27	27	0	27	0
	Plasma (heparin)	27	27	0	27	0
	Plasma (citrate)	27	27	0	27	0
	Whole Blood	27	27	0	27	0
Negative	Serum	27	0	27	0	27
	Plasma (EDTA)	27	0	27	0	27
	Plasma (heparin)	27	0	27	0	27
	Plasma (citrate)	27	0	27	0	27
	Whole Blood	27	0	27	0	27

8. Precision

Product precision was determined by testing 6 replicates of 3 serum or 3 whole blood specimens containing different concentrations of HBsAg.
Within-run precision of 100% was observed for both specimen matrices.
Between-day precision was determined over 5 different days, and observed as 100% for both serum and whole blood.
Between-lot precision was determined with 3 lots of devices. 100% precision was observed for both serum and whole blood.
Between-operator precision was determined by 3 operators.100% precision was observed for both serum and whole blood.

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of HBsAg in serum, plasma or whole blood. Failure to follow the procedure may lead to inaccurate results.
2. The *OnSite* HBsAg Combo Rapid Test is limited to the qualitative detection of HBsAg in human serum, plasma or whole blood. The intensity of the test line does not correlate with the antibody titer of the specimen.
3. Specimens that produce very faint lines (indeterminate) should be re-tested with two new devices or an alternative method(s). However, a non-reactive or negative test result does not preclude the possibility of exposure to or infection with HBV.
4. A negative or non-reactive result can occur if the concentration of HBsAg present in the specimen is below the level detectable by the assay or HBsAg was not present during the stage of disease in which a sample was collected.
5. The presence of hepatitis B surface antibodies (anti-HBs) may interfere with test results from HBsAg-positive specimens.
6. Infection may progress rapidly. If symptoms persist while the result from the *OnSite* HBsAg Combo Rapid Test is negative or non-reactive, it is recommended to test with an alternative test method.
7. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
8. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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Description of Symbols Used

	Consult instructions for use or Consult electronic instructions for use	EC REP	Authorized Representative in the European Community / European Union		Contains sufficient for <n> tests
REF	Catalog number	IVD	*In vitro* diagnostic medical device		Use-by-date
	Temperature limit	LOT	Batch code		Do not re-use
	Manufacturer		Date of manufacture	CE	CE marking

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Date released: 2022-06-15
English version

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