FIRST RESPONSE® HBsAg CARD TEST

One Step Hepatitis B Detection Card Test

For detection of Hepatitis B surface antigen in human whole blood/serum/plasma

REF PI10FRC25,PI10FRC30,PI10FRC50, PI10FRC60 & PI10FRC100

4°C 30°C IVD

Intended Use:

First Response® HBsAg Card Test is a chromatographic immunoassay for qualitative detection of the Hepatitis B surface antigen in serum/plasma/whole blood (venous & capillary blood) specimens. It is intended for use in medical institution as a aid for diagnosis and management of patients related to the infection with Hepatitis B as well as for primary screening of blood from volunteer donors on the spot.

Introduction:

Hepatitis B virus is a double stranded DNA virus, consist of an outer lipid envelope and an isohedral nucleocapsid core composed of protein. The virus belonging to family Hepadnaviridae. Eight major Genotypes (A-H) and series of 4 serotypes (adr, adw, ayr, ayw) has been identified. Hepatitis B virus was discovered in 1965 by Baruch Samuel Blumberg. The disease is characterized by acute illness causing liver inflammation, vomiting, Jaundice and rarely death. Chronic forms may cause cirrhosis and liver cancer. D.S. Dane and others discovered the virus particle in 1970 by electron microscopy and in early 1980 the genome of the virus has been sequenced and first vaccine were tested. Hepatitis B surface antigen is most frequently used to screen for the presence of this infection. The infectious virion contain an inner core particle enclosing viral genome. Hepatitis B is a viral infection that attacks the liver and can cause both acute and chronic disease. The virus is transmitted through contact with the blood or other body fluids of an infected person. Two billion people worldwide have been infected with the virus and about 600 000 people die every year due to the consequences of Hepatitis B. The hepatitis B virus is 50 to 100 times more infectious than HIV.

Assay Principle:

First Response® HBsAg Card Test is based on principle of immunochromatography in which nitrocellulose membrane is pre-coated with monoclonal antibodies (test line) specific to Hepatitis B surface antigen in whole blood/serum/plasma samples. When the test sample flows through the nitrocellulose membrane, second monoclonal antibodies specific for Hepatitis B surface antigen conjugated with colloidal gold, binds to Hepatitis B surface antigens in the whole blood/serum/plasma/ samples. This antigen-conjugated antibody complex moves through the nitrocellulose membrane and binds to the immobilized Hepatitis B surface antigen specific monoclonal antibodies at the test line, which leads to the formation of colour line indicating reactive results. The control line will appear irrespective of reactive or non reactive sample.

Material Provided	PI10FRC25	PI10FRC30	PI10FRC50	PI10FRC60	PI10FRC100
Test Device Pouch Containing: 1 Test Device, 1 Desiccant	25 Nos.	30 Nos.	50 Nos.	60 Nos.	100 Nos.
Specimen Transfer Device	25 Nos.	30 Nos.	50 Nos.	60 Nos.	100 Nos.
Assay Buffer Bottle	1 No.	1 No.	2 Nos.	4 Nos.	4 Nos.
Sterile Single-use Lancets	25 Nos.	30 Nos.	50 Nos.	60 Nos.	100 Nos.
Alcohol Swabs	25 Nos.	30 Nos.	50 Nos.	60 Nos.	100 Nos.
Instructions for use	1 No.	1 No.	1 No.	1 No.	2 Nos.

Materials Required but Not Provided

- New pair of disposable gloves
- Pen
- Timer
- Extra lancets and alcohol swabs, if needed
- Sharp disposable box
- Venipuncture blood collection materials and precision pipette plus tips (if whole blood is collected by venipuncture)
- Biohazardous waste container

Storage and Stability:

First Response® HBsAg Card Test should be stored at 4-30°C. Do not freeze the kit or components. Assay Buffer (opened & unopened) & the unopened Test Device are stable until the expiry date printed on the label, when stored at room temperature 4-30°C. The test device is sensitive to humidity and heat. Perform the test immediately after removing the Test Device from the foil pouch. The shelf life of the kit is as indicated on the outer package. Do not use Test Device and Assay buffer beyond the date of expiry.

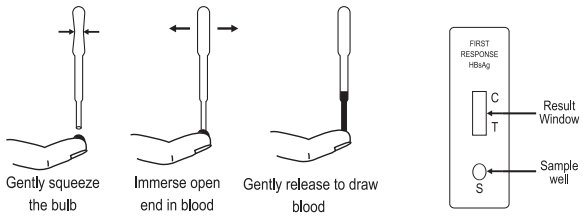
Precautions:

1) For in vitro diagnostic use only.
2) Test Devices and Assay Buffer of different lot must not be used.
3) Do not use the Test Device if the pouch is not intact.
4) Do not use the Lancet if the seal is broken.
5) Check the desiccant for saturation, immediately after opening the pouch.
6) Do not smoke, eat or drink while handling specimens and performing a test.
7) The test device, alcohol swab, lancet and sample pipette are intended for single use only.
8) Follow the assay procedure strictly, deviation will invalidate the test results.
9) Perform the test by using kit assay Buffer, any other Buffer or fluid will invalidate the test results.
10) Do not touch the tip of Assay Buffer bottle, it might contaminate Assay Buffer.
11) Wear protective gloves while handling specimens. Dispose of used gloves as biohazard waste. wash hands thoroughly afterwards.
12) Avoid splashing or aerosol formation.
13) Clean up spills thoroughly using an appropriate disinfectant.
14) Decontaminate and dispose of all used specimens, test devices, alcohol swabs and Specimen Transfer device as a infectious waste, in a biohazardous waste container. Dispose of used lancets in a sharp box.

Specimen Collection and Storage:

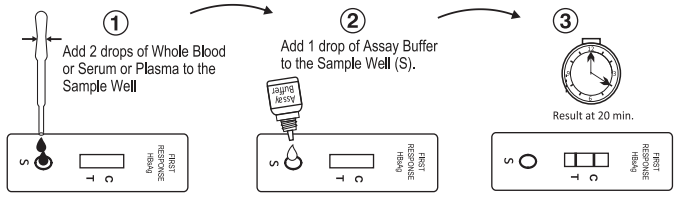
1) Capillary blood collection: Clean the area to be lanced with alcohol swab and allow it to air dry. Creates more interstitial fluid and pierce with sterile lancet. Take 2 to 3 µl specimen transfer device provided, immerse the open end in the blood sample and release the pressure to draw blood into the specimen transfer device.
2) Venous Blood collection: Collect the Whole Blood in collection tubes containing anticoagulants like EDTA, Heparin or Sodium Citrate by venipuncture.
3) Plasma collection: Collect the Whole Blood in collection tubes containing anticoagulants like EDTA, Heparin or Sodium Citrate by venipuncture. Centrifuge it at 3000 rpm for 10-15 minutes to obtain Plasma.
4) Serum: Collect Whole Blood in collection tubes without having any anticoagulants by venipuncture. Keep it in standing position for 30 min and cetrifuge it at 3000 rpm for 10-15 minutes to obtain serum.
5) Whole Blood specimen may be used for testing immediately or may be stored at 2-8°C for up to 3 days.
6) If Serum or Plasma specimens are not immediately tested, they should be refrigerated at 2-8°C. For storage periods greater than three days, freezing at -20 °C is recommended. They should be brought to room temperature prior to use.
7) Serum or Plasma specimens containing precipitate may yield inconsistent test results. Such specimens must ALWAYS be centrifuged prior to assaying.

Specimen Transfer Device



Test Procedure

1) Ensure that the Test Device is at room temperature before starting the procedure.
2) Take the test device and the specimen transfer device from the kit. Label the Test Device with the patient Identification Number / Name. Place the test device on a flat, clean and dry surface.
3) Slowly add 50 µl (Two drops) of Whole Blood / serum/ plasma to the sample well (S) using the Specimen transfer device. Dispose of used specimen transfer device as biohazard waste.
4) Add One Drop of the Assay Buffer to the sample well (S).
5) Observe for development of colored lines in the Result Window.
6) Interpret test results at 20 minutes. (After recording the results, dispose of test device as a biohazard waste).
7) Do not interpret after 20 minutes.

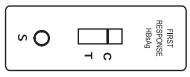


Pictorial Presentation of Test Procedure

Interpretation of the Test

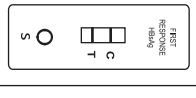
Non-reactive Result

If only one color line appear, at control line 'C' as in the figure, the specimen is negative.



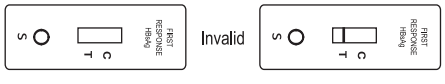
Reactive Results

If two color lines appears, one at control line 'C' and other at test line 'T' as in the figure, the specimen is reactive for Hepatitis B surface antigen.



Invalid Result

If no color line appears at the control line 'C' within the stipulated time then the result is invalid.
The invalid test result should be retested with new test device.



Limitations:

1) The following anticoagulants have been validated for use with this test: Heparin, EDTA & Sodium citrate.
2) Hemolytic sample may give reddish background even after end of test time.
3) Interpret faint line as a reactive line. Repeat the test in case of very faint test line or if have any doubt for test line.
4) False negative results may arise because of hook effect due to very high Hepatitis surface antigen specimens.

5) The First Response® HBsAg Card Test is for in-vitro diagnostic use only. The test is designed for use with serum or plasma or whole blood specimens only. Use of other body fluids, including Urine or saliva has not been established.
6) This test will indicate only the presence or absence of HBsAg in the specimen, and should not be used as the only basis for the diagnosis of hepatitis viral infection. As with all diagnosis tests, results must be considered along with other clinical information available to the physician. If symptoms persist additional follow- up testing using other clinical methods is required. A negative at any point does not preclude the possibility of Hepatitis B infection.

Performance Characteristics:

(A) Clinical Specificity and Sensitivity

(A.1) In-House Evaluation:

First Response® HBsAg Card Test have been tested using an in-house panel of Positive and Negative clinical samples confirmed by a leading commercial anti HBsAg ELISA and RDT Kit. The result shows that First Response® HBsAg Card Test is very accurate compared to other commercial ELISA and RDT kit. In a comparison of the First Response® HBsAg Card Test versus a leading commercial anti HBsAg ELISA and Rapid test, results gave sensitivity of 100%, A specificity of 100% and total agreement of 100%.

Reference		Performance		First Response HBsAg Card Test		Total Results
Method	Results	Sensitivity	Specificity	Positive	Negative	
Commercial ELISA	HBsAg Positive	100%	0	150	00	150
	Negative Samples	0	100%	00	620	620
Total Results		100%	100%	150	620	770

Reference		First Response® HBsAg Card Test		Total Results	95% Confidence interval
Method	Specimen	Positive	Negative		
Elisa/RDT by Commercial available	Sensitivity	150	00	150	(96.89% - 100%)
	Specificity	00	620	620	(99.23% - 100%)

(B) Analytical Specificity and Sensitivity:

(B.1) Analytical Specificity:

(B.1.1) Potential Interfering Substances:

The interfering substances that may affect performance of the First Response® HBsAg Card Test are tested with kit. The results are presented in following tables and demonstrated that, the substances studies does not interfering performance of the First Response® HBsAg Card Test. **However, hemolysed samples and lipemic samples showed poor back ground clearance, hence not recommended to use it for testing. The lipemic samples can be use for the testing after centrifugation.

Sample Details	Sample size	HBsAg Reactivity	Sample Details	Sample size	HBsAg Reactivity
Lipemic samples**	05	Negative	Low Hematocrit samples	05	Negative
Icteric samples	05	Negative	Whole blood specimen in ACD anticoagulant	05	Negative
Hemolytic samples**	05	Negative	RF Ab 4001-5000 IU/mL Plasma	08	Negative
High Hematocrit samples	05	Negative	dsDNA Antibody Positive Plasma	01	Negative

(B.1.2) Cross Reactivity Study:

First Response® HBsAg Card Test is tested with other diseases/conditions, which may give cross reactivity with Test. The results are mentioned in following table and demonstrated that First Response® HBsAg Card Test showed no cross reactivity with studied diseases/conditions samples.

Sample Details	Sample size	HBsAg Reactivity
HIV -1 Positive Serum	202	Negative
HIV-2 Positive Serum	33	Negative
HCV Positive Serum	50	Negative
Syphilis Positive Serum	71	Negative

(B.2) Analytical Sensitivity

(B.2.1) Analytical Sensitivity – In – House Evaluation

The Analytical Sensitivity of the First Response® HBsAg Card Test is determined by testing commercially available seroconversion panels. A commercially available CE-marked rapid lateral flow test is used as a reference test for the comparative performance study. The HBsAg seroconversion sample study is done as specified in CTS 2009/886/EC for In Vitro Diagnostics Medical Device. A total of 30 seroconversion panels were tested by the manufacturer (30 panels by internal evaluation) to meet the criteria specified in CTS 2009/886/EC for In Vitro Diagnostics Medical Device.

Analytical Sensitivity – In – House Evaluation							
Total Seroconversion Panels	Total Specimens	First Response HBsAg Card Test			Reference CE-marked rapid lateral flow test.		
		Positive	Negative	Detection Index**	Positive	Negative	Detection Index**
30	340	69	271	0.20	44	296	0.12
0.2 IU Working Standard	2	2	0	1.0	2	2	1.0

** **Detection Index =** Total number of positive sample by test kit / Total number of sample

Precision:

a) Within-run precision was determined by using ten replicates of four different specimens containing different concentrations of antigen. Within-run precision was observed as 100%.
b) Between operator and sites precision was determined by using six replicates of ten different specimens containing different concentrations of antigen at two different sites and by two different operators. The test showed 100% precision at both sites with both operators.
c) Between-run precision was determined by using four different specimens containing different concentrations of antigen in three different replicates with three different lots of test devices. Between-run precision was observed as 100%.

SYMBOL LEGENDS

Symbol	Explanation of symbol	Symbol	Explanation of symbol
	Consult instructions for use		Contains sufficient for < n > tests
	Non Sterile	REF	Product Code
IVD	In vitro diagnostic medical device	LOT	Lot Number
4°C 30°C	Store at 4-30 °C		Manufacturer
	Caution		Date of manufacture (YYYY-MM)
	Keep dry		Expiration Date (YYYY-MM)
	Do not reuse		

References:

1. Chi-Jen Chu and Anna S. F. Lok (2002). Clinical Significance of Hepatitis B Virus Genotypes. Hepatology, 35:922-929.
2. Dienstag JL (2008). Hepatitis B virus infection. The New England Journal of Medicine, 359:1486-1500.
3. Zhihua Liu, and Jinlin Hou (2006). Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) Dual Infection. International Journal of Medical Sciences, 3(2):57-62.
4. World Health Organization (2009). Hepatitis B fact sheet. http://www.who.int/mediacentre/factsheets/fs204/en/index.html

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• ISO 13485 & EN ISO 13485 Certified Company

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Note : Instructions for use will be printed in local language of the country using the test, if required.