Infection

ichroma™ HBsAg

INTENDED USE

ichroma™ HBsAg is a fluorescence Immunoassay (FIA) for the qualitative determination of HBsAg in human whole blood/serum/plasma. It is useful as an aid to diagnosis of Hepatitis B virus infection.

For *in vitro* diagnostic use only.

INTRODUCTION

The hepatitis B virus (HBV) is responsible for hepatic lesions, as in fulminant acute hepatitis or chronic hepatitis that can result in cirrhosis and hepatocellular carcinoma. Detection of the Hepatitis B virus surface antigen (HBsAg) in serum or plasma indicates an infection caused by the hepatitis B virus. It is the first marker to appear during the course of the disease.[1] Clinical and biological symptoms appear two to three weeks after the initial infection with HBV. Presence of HBsAg can be as short as a few days or as long as several years. If HBsAg persists for more than six months, the hepatitis is classified as 'chronic'. Due to existence of numerous asymptomatic chronic carriers, screening for HBsAg is required for each blood donation and for each pregnancy to enable the newborns of the carrier mother to receive prophylactic treatment.[2),3)]

PRINCIPLE

The test uses a sandwich immunodetection method; the detector antibodies in buffer bind to antigens in the sample, forming antigen-antibody complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized-antibody on the test strip.

More antigens in the sample will form more antigen-antibody complexes, which leads to stronger fluorescence signal by detector antibodies, which is processed by instrument for ichroma tests to display the 'Positive' / 'Negative' / 'Indeterminate' in the sample.

COMPONENTS

ichroma™ HBsAg consists of 'cartridges', 'detector tubes', 'detector diluent'.

- The cartridge contains the membrane called a test strip which has anti human HBsAg and recombinant HBsAg at the test line, and chicken IgY at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant in a box.
- The detector tube has 2 granules containing anti human HBsAg-fluorescence conjugate, anti-chicken IgY-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in phosphate buffered saline (PBS). All detector tubes are packed in an aluminum foil pouch.
- The detector diluent contains bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in PBS, and it is pre-dispensed in a vial. The detector diluent is packed in a box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, detector tube, detector diluent, and ID chip) must match each other.
- Do not interchange test components between different lots or use test components after the expiration date, either of which might yield incorrect test result(s).
- Do not reuse cartridges or detector tubes. A cartridge should be used for testing one sample only. A detector tube should be used for processing of one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use the cartridge, if pouch is damaged or has already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with local regulation. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- Allow cartridge, detector tube, detector diluent and the sample to be at room temperature for approximately 30 minutes before use.
- The instrument for ichroma™ tests may generate slight vibration during use.
- Used cartridges, detector tubes, detector diluent and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **ichroma™ HBsAg** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ HBsAg** should be used only in conjunction with instrument for ichroma™ tests.
  - Have to use recommended anticoagulant sample.

recommended anticoagulant
Sodium Heparin, Lithium Heparin, EDTA K_2 EDTA, K_3 EDTA, Sodium citrate

STORAGE AND STABILITY

	Storage condition		
Component	Storage Temperature	Shelf life	Note
Cartridge	4 - 30 °C.	20 months	Disposable
Detector tube	2 - 8 °C	20 months	Disposable
Detector diluent	2 - 8 °C	20 months	Disposable

- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result(s) due to the non-responsiveness of the antigen to the antibodies which is the most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may also cause false negative result as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-29

Components of **ichroma™ HBsAg**

- Cartridge Box:
  - Cartridge 25
  - ID Chip 1
  - Instruction for Use 1
- Buffer Box
  - Detector tube 25
  - Detector diluent 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ HBsAg**.

Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
- **Printer** REF FPRR007
- **Boditech HBsAg Control** REF CFPO-142

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ HBsAg** is human whole blood/serum/plasma.

- It is recommended to test the sample within 24 hours after collection.
- The serum or plasma should be separated from the clot by centrifugation within 3 hours after the collection of whole blood sample.
- Samples may be stored for a week at 2-8 °C prior to being tested. If testing will be delayed more than a week, samples should be frozen at -20 °C.
- Samples stored frozen at -20 °C for 2 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- Once the sample was frozen, it should be thawed one time and only for test, because repeated freezing and thawing can result in the changed test values.

TEST SETUP

- Check the contents of **ichroma™ HBsAg**: Sealed Cartridges, Detector tubes, Detector diluent, ID Chip, and an Instruction for use.
- Ensure that the lot number of the cartridge matches that of the detector tube, the detector diluent as well as the ID chip.
- If the sealed cartridge, detector tube and detector diluent have been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing
- Turn on the Instrument for ichroma™ tests.
  (Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)

TEST PROCEDURE

< Multi mode >

1) Open the diluent vial and transfer 75 µL of diluent buffer using a pipette to the detector tube. When the granule form is completely dissolved in the tube, it becomes detection buffer
2) Transfer 75 µL of sample (whole blood/serum/plasma/control) using a pipette to the detector tube.
3) Mix well by pipetting 10-20 times. (The sample mixture must be used immediately.)
4) Pipette out 75 µL of a sample mixture and dispense it into the sample well on the cartridge.
5) Leave the sample-loaded cartridge at room temperature for 12 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*
6) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
7) Tap the 'START' icon on the screen.
8) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
9) Read the test result on the display screen of the instrument for ichroma™ tests.

< Single mode >

1) Open the diluent and transfer 75 µL of detector diluent buffer using a pipette to the detector tube. When the granule form is completely dissolved in the tube, it becomes detection buffer.
2) Transfer 75 µL of sample (whole blood/serum/plasma/control) using a pipette to the detector tube.
3) Mix well by pipetting 10-20 times. (The sample mixture must be used immediately.)
4) Pipette out 75 µL of a sample mixture and dispense it

into the sample well on the cartridge.

5) Inserting the device into the holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
6) Tap the 'START' button on the instrument for ichroma™ tests.
7) Cartridge goes inside the Instrument for ichroma™ tests and will automatically start scanning the sample-loaded cartridge after 12 minutes.
8) Read the test result on the display screen of the instrument for ichroma™ tests.

INTERPRETATION OF TEST RESULT

- Instrument for ichroma™ tests calculates the test result automatically and displays "Positive"/"Negative"/ "Indeterminate".
- Ancillary value is served in the form of a cut-off index (COI).

Cut-off index (COI)	Result	Note
≤ 0.90	Negative for HBsAg	No need to additional test.
> 0.90, < 1.0	Indeterminate.	Need to retest.
≥ 1.0	Positive for HBsAg	Need to confirmation test.

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are provided on demand with **ichroma™ HBsAg**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical Sensitivity**

- Cut-off
  ichroma™ HBsAg decides between positive and negative through COI calculated by ichroma™ II algorism.

Cutoff Index (COI)	Result
COI ≥ 1.0	Positive
0.90 < COI < 1.0	Indeterminate
COI ≤ 0.90	Negative

- Seroconversion panel

Sero-conversion panel	Roche Cobas e411 Roche Elecsys HBsAg II		ichroma™ HBsAg		Total samples
	Positive	Negative	Positive	Negative	
HBV 6285	6	10	6	10	16
HBV 11005	3	10	3	10	13
HBV 6293	3	4	3	4	7
HBV 6273	2	4	2	4	6
HBV 6287	2	9	2	9	11
HBV 11002	2	4	2	4	6
HBV 11004	3	5	3	5	8
HBV 11059	5	4	5	4	9
HBV 11064	2	7	2	7	9
HBV 9072	7	10	7	10	17
Total	35	67	35	67	102

- **Analytical Specificity**

- Cross-reactivity
  There was no significant cross reactivity from these material with the **ichroma™ HBsAg** test.

Clinical category	ichroma™ HBsAg results		
	Number of samples	Negative	Positive
CMV	19	19	0
EBV	20	20	0
HAV	28	28	0
HCV	10	10	0
HSV	19	19	0
Rubella	20	20	0
VZV	20	20	0
Syphilis	17	17	0
ANA	23	23	0
RF	23	23	0
Samples of pregnant women	39	39	0
Total	238	238	0

- Interference
  There was no significant interference from these material with the **ichroma™ HBsAg** test.

Materials	Concentration
Heparin	100,000 U/L
EDTA	5 μM
Sodium citrate	0.085 M
Bilirubin	500 μM
Hemoglobin	2 g/L
Triglycerides	1.5 mg/mL
Cholesterol	20 mM
Albumin	60 mg/mL

■ **Precision**

- Between lot
  One person tested three different lots of **ichroma™ HBsAg**, ten times at each concentration of the control standard.
- Between person
  Three different persons tested same LOT of **ichroma™ HBsAg**, five times at each concentration of the control standard.
- Between day
  One person tested one LOT of **ichroma™ HBsAg** during three days, five times at each concentration of the control standard.
- Between site
  One person tested one LOT of **ichroma™ HBsAg** in three different space, five times at each concentration of the control standard.

HBsAg Cal	Between lot		Between person	
	Positive /Number of tests	Positive rate	Positive /Number of tests	Positive rate
Negative	0/10	0 %	0/5	0 %
High	10/10	100 %	5/5	100 %
Mid	10/10	100 %	5/5	100 %
Low	10/10	100 %	5/5	100 %

HBsAg Cal	Between day		Between site	
	Positive /Number of tests	Positive rate	Positive /Number of tests	Positive rate
Negative	0/5	0 %	0/5	0 %
High	5/5	100 %	5/5	100 %
Mid	5/5	100 %	5/5	100 %
Low	5/5	100 %	5/5	100 %

■ **Comparability with reference product**

Test product	Sample group	Negative	Positive	Number of samples
	Number of samples	560	109	669
Roche Elecsys HBsAg II	Negative	560	0	560
	Positive	0	109	109
ichroma™ HBsAg	Negative	558	1	559
	Positive	2	108	110
Compatibility (%)		99.6	99.1	

REFERENCES

1. Performance evaluation of immunoassay detection of HBsAg mutants and their clinical significance in the high risk groups. Jung-in Choi et al., Lab Med Online., 2013, 3:88-96
2. Centers for Disease Control and Prevention. Recommendations of the Immunization Practices Advisory Committee Prevention of Perinatal Transmission of Hepatitis B virus: Prenatal Screening of all Pregnant Women for Hepatitis B Surface Antigen. MMWR, 1988; 37:341-355
3. Centers for Disease Control and Prevention. Hepatitis B virus: A comprehensive strategy for eliminating transmission in the united states through universal childhood vaccination: recommendations of the immunization practices advisory committee (ACIP), MMWR, 1991; 40:1-19

Note: Please refer to the table below to identify various symbols.

Σ	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse

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IVD