If one of the Negative Control wells has an absorbance more than 0.10 O. D. above the mean of two, discard that value and calculate the new Negative Control mean from two remaining replicates.

Cut-off value

Calculate the Cut-off value by adding 0.10 to the mean of the Negative Control replicates.

Mean Negative Control = 0.010

Cut-off value = 0.010 + 0.100 = 0.110

QUALITY CONTROL

Results of an assay are valid if the following criteria for the controls are met:

Negative Control

The mean absorbance must be less than 0.10.

Positive Controls

The absorbance of each of the Positive Controls should be more than 1.0

Assays which do not meet these criteria should be repeated. In the unlikely event of the results repeatedly failing to meet either the Quality Control criteria or the expected performance of the test, please contact your representative.

INTERPRETATION OF RESULTS

Non-reactive Results

Samples giving an absorbance less than the Cut-off value are considered negative in the assay.

Reactive Results

Samples giving an absorbance equal to or greater than the Cut-off value are considered initially reactive in the assay.

Note: Samples which are found reactive should be retested in duplicate using the original source. Samples that are reactive in at least one of the duplicate retests are considered repeatedly reactive in Merilisa HBsAg and are presumed to contain HBsAg. Such samples should be further investigated and the presence of HBsAg confirmed by other tests. Samples that are non-reactive i.e. with an absorbance less than that of the Cut-off value, should be considered non-reactive for HBsAg.

LIMITATIONS OF PROCEDURE

1. The **Test Procedure** and **Interpretation of Results** must be followed.
2. This test has only been evaluated for use with individual serum, EDTA plasma or citrate plasma samples. Merilisa HBsAg has not been evaluated for any other purpose.
3. A negative result with an antibody detection test does not preclude the possibility of infection.
4. Non-repeatable reactive results may be obtained with any EIA procedure.
5. The most common sources of error are: a) Imprecise delivery of Sample, Conjugate or Substrate into the wells. b) Contamination of Substrate with Conjugate. c) Contamination with conjugates from other assays. d) Blocked or partially blocked washer probes. e) Insufficient aspiration leaving a small volume of Washing solution in the wells. f) Failure to ensure that the bottom surface of the wells is clean and dry, and that no air bubbles are present on the surface of the liquid in the wells before a plate is read. g) Failure to read at the correct wavelength or use of an incorrect reference wavelength.
6. The use of highly haemolysed samples, incompletely clotted sera, plasma samples containing fibrin or samples with microbial contamination may give rise to erroneous results.

SPECIFIC PERFORMANCE CHARACTERISTICS

The performance of Merilisa HBsAg has been determined by in-house testing of 760 samples, Merilisa HBsAg assay demonstrated a specificity of 100 % and a sensitivity of 100%.

Merilisa HBsAg demonstrated the **analytical sensitivity of 0.4 PEIU/ml.**

BIBLIOGRAPHY

1. Blumberg, B.S., Sutnick, A.I., London, W.T., (1968) Hepatitis and leukemia: their relation to Australia antigen. Bull. N.Y. Acad. Med.; 44(12): 1566-1586.
2. Taylor, R.N., Fulford, K.M., (1976) Results of the Center for Disease control Proficiency Testing Program for the detection of hepatitis B surface antigen. J Clin Microbiol.; 4(1): 32-39.
3. Scheiblauer, H., El-Nageh, M., Diaz, S., Nick, S., Zeichhardt, H., Grunert, H.P., Prince, A.,(2009) Performance evaluation of 70 hepatitis B virus (HBV) surface antigen (HBsAg) assays from around the world by a geographically diverse panel with an array of HBV genotypes and HBsAg subtypes. Vox Sang.; 98: 403-414.

QA0I -701.09
Rev 00, Dt. 01/04/2013

Symbols used on Meril Diagnostics labels:

REF	Catalogue No	IVD	In Vitro Diagnostics
LOT	Batch No		Manufacturing Date
	Expiry Date		Consult Instruction for Use
	Manufacturer		Storage temperature

Meril Diagnostics

MERILISA HBsAg

Enzyme immunoassay for detection of HBsAg in human serum or plasma

For in vitro diagnostic use
Read this pack insert thoroughly before use

Product Code: HPBELI-01

INTENDED USE

MERILISA HBsAg is Enzyme immunoassay for the qualitative determination of Hepatitis B surface antigen in human serum or plasma by healthcare professional.

SUMMARY AND EXPLANATION OF THE TEST

Hepatitis B is a disease caused by a viral infection. Throughout the various serological markers appear infection among which is the HBsAg. In 1964, Blumberg et al. first detected HBsAg in the serum of an Australian Aboriginal an antigen reacted with an antibody serum from a haemophiliac patient New York. Hepatitis B virus (HBV) is an enveloped DNA virus. During infection, HBV produces an excess of hepatitis B surface antigen (HBsAg), which can be detected in the blood of infected individuals. HBsAg is the first serological marker after infection with HBV appearing one to ten weeks after exposure and two to eight weeks before the onset of hepatitis. HBsAg persists during this acute phase and clears late in the convalescence period. Failure to clear HBsAg within six months indicates a chronic HBsAg carrier state.

Merilisa HBsAg employs HBsAg specific antibody and is expected to detect HBsAg in human serum or plasma. Consequently potentially infectious samples of serum, EDTA plasma or citrate plasma can be identified.

PRINCIPLE OF THE PROCEDURE

Merilisa HBsAg is based on microwells coated with monoclonal anti-HBsAg Antibody. The Conjugate is polyclonal anti-HBsAg antibody labelled with horseradish peroxidase.

Samples and controls are incubated in the wells and HBsAg if present bind to the monoclonal anti-HBsAg antibody on the microwell. In a subsequent step, Conjugate is added which in turn binds to any specific antigen already bound to the antibody on the well. Unbound Conjugate is washed away and a solution containing 3, 3', 5, 5'-tetramethylbenzidine (TMB) and hydrogen peroxide is added to the wells. Wells with bound Conjugate develop a blue to bluish green colour which is converted to an yellow to orange colour when the reaction is stopped with sulphuric acid. After incubation the reactions are stopped with sulphuric acid and the colour is read spectrophotometrically. The intensity of colour produced in the wells is directly proportional to the concentration of HBsAg in the sample. Wells containing negative samples remain colourless.

REAGENTS

DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

1. HBsAg Ab Coated Microplate

One plate of 96 microwells coated with monoclonal anti-HBsAg antibody. If less than the whole plate is being used allow the wells to reach room temperature (18 to 30°C) before removal from the bag. Place unused wells in the sealable storage bag provided and return to 2 to 8°C. Once opened, microwells should be used within one month.

2. Negative Control

Vial containing 1.0 ml of normal human serum with preservative. Negative control has been tested and found negative for anti-HIV 1+2, HBsAg, Anti-HCV and Syphilis.

3. Positive Control

Vial containing 1.0 ml of inactivated human serum in a buffer containing protein with preservative. Positive control has been tested and found negative for Anti-HIV 1+2, Anti-HCV and Syphilis.

4. Washing Solution (20X)

Bottle containing 20 times working strength Phosphate Buffer Saline Wash Solution with detergent. Add one volume of Washing Solution Concentrate to 19 volumes of distilled or deionised water to give the required volume. If the Crystals are observed in the Washing Solution (20X), dissolve crystals by keeping Washing Solution (20X) at 37°C until the crystals dissolves. Store the diluted Washing Solution at 18 to 30°C in a closed vessel under which conditions it will retain activity for one month.

5. Conjugate (51X)

Vial containing polyclonal Anti-HBsAg conjugated to HRP with protein stabilizers and preservatives. Bottle containing 51 times working strength conjugates. Add one volume of Conjugate Concentrate to 50 volumes of Conjugate Diluent to give the required volume.

6. Conjugate Diluent

Bottle containing solution consisting of buffer, bovine protein, preservatives and detergent.

Meril Diagnostics Pvt. Ltd., Survey No. 135/2/B, Muktanand Marg, Chala, Vapi – 396 191, Gujarat, India.
Tel: +91 22 39350700; Fax: +91 22 39350777 **E:** diagnostics@merillife.com; **W:** www.merillife.com

Preparation Working conjugate solution

Dilute Conjugate (51X), 1:50 with Conjugate Diluent as per **Table1**.

Table 1:

No. of Strips	1	2	4	6	8	10	12
Conjugate Diluent, mL	0.5	1	2	3	4	5	6
Conjugate (51X), µL	10	20	40	60	80	100	120

Store the diluted Conjugate at 2 to 8°C in a closed vessel under which conditions it will retain activity for 48 hours.

7. Substrate Solution

Bottle containing colourless solution of 3, 3', 5, 5' tetramethylbenzidine and hydrogen peroxide and stabilizers.

8. Stop Solution

Bottle containing colourless solution of diluted mineral acid and stabilizers.

WARNINGS AND PRECAUTIONS

The reagents are for *in vitro* diagnostic use only.

For professional use only.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

SPECIMEN COLLECTION

Serum, EDTA plasma or citrate plasma samples may be used. Ensure that the serum samples are fully clotted. Remove any visible particulate matter from the sample by centrifugation.

SPECIMEN TRANSPORT AND STORAGE

Store the samples at 2 to 8°C. Samples not required for assay within 7 days should be stored frozen (-15°C or colder). Avoid multiple freeze-thaw cycles. After thawing ensure samples are thoroughly mixed before testing.

MATERIALS REQUIRED BUT NOT PROVIDED

1. **Freshly distilled or high quality deionised water** is required for dilution of Washing Solution for use in conjunction with automated washers.
2. **Calibrated Micropipettes and Multichannel micropipettes** of appropriate volume.
3. **Incubator** capable of maintaining the temperature limits required as per assay protocol.
4. **Instrumentation**
   a. Automated microplate strip washer.
   b. Microplate reader or Fully automated microplate processor.
   c. All instruments must be validated before use.
   d. Please contact your representative for details of recommended systems, software protocols for instrumentation and validation procedures.
5. **Disposable Reagent Troughs**.
6. **Sodium hypochlorite** for disposal of hazardous substance or remnants of the assay.

PRECAUTIONS

1. Potentially contaminated materials should be disposed of safely according to local requirements.
2. Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated area swabbed with, for example, 1.0% sodium hypochlorite before work is continued. Sodium hypochlorite should not be used on acid-containing spills unless the spill area is first wiped dry. Materials used to clean spills, including gloves, should be disposed of as potentially biohazardous waste. Do not autoclave materials containing sodium hypochlorite.
3. Neutralised acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.
4. Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
5. The following reagents contain low concentrations of harmful or irritant substances: a) The Conjugate Diluent and Sample Diluent contain ProClinR300 which can be absorbed through the skin and is a sensitising agent.
6. Sulphuric acid used in Stop Solution is corrosive and should be handled with appropriate care. If either come into contact with the skin or eyes, wash thoroughly with water.
7. If any of the reagents come into contact with the skin or eyes wash the area extensively with water.
8. Do not use the reagents beyond the stated expiry date.
9. Follow Good Laboratory Practice to avoid microbiological contamination of reagents as this may reduce the life of the product and cause erroneous results.
10. Do not modify the **Test Procedure** or substitute reagents from other manufacturers or other lots unless the reagent is stipulated as interchangeable. Do not reduce any of the recommended incubation times.
11. Allow all reagents and samples to come to 18 to 30°C before use. Immediately after use return reagents to the recommended storage temperature.
12. Do not expose reagents to strong light or hypochlorite fumes during storage or during incubation steps.
13. Do not allow wells to become dry during the assay procedure.
14. Do not cross-contaminate reagents. It is recommended to use dedicated separate pipettes for use with the Substrate Solution and Conjugate.
15. Do not touch or splash the rim of the well with Conjugate. Do not blow out from micropipettes; reverse pipetting is recommended whenever possible.
16. Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate.
17. Do not contaminate microwells with dust from disposable gloves.
18. Ensure the assay is run within the recommended temperature limits in the assay protocol.
19. Do not use CO_2 Incubators.
20. Do not store the Stop Solution in a shallow dish or return it to a stock bottle after use.
21. The possibility of cross contamination between assays needs to be excluded when validating assay protocols on instrumentation.

TEST PROCEDURE

Step 1: Prepare working **Conjugate solution** and **Washing solution**.

Step 2: Use only the number of wells required for the test. Avoid touching the tops or bottoms of the wells.

Step 3: Add 50 µl of **Samples and Controls** to the wells.

For each plate use the first column of wells for the assay Controls. Add the Controls to the designated wells after dispensing the samples. Pipette 50 µl of the Negative Control into each of three wells B1 to D1 and 50 µl of the Positive Control into wells E1 and F1 respectively. Do not add anything in Blank well (A1).

Use of a white background will aid visualisation of sample addition.

Step 4: Add 50 µl of **Conjugate** to all wells except blank well (A1).

Step 5: Cover the wells with adhesive strip(s) and **incubate** for **60 mins** at 37°C ± 1°C.

Step 6: At the end of the incubation, discard the content of the plate. Aspirate the contents of the wells and fill them completely (approximately 350 µl) with the diluted washing solution. Repeat the process of aspiration and washing **4 more times**. Ensure that each column of wells soaks for at least 30 seconds before the next aspiration cycle. After the last washing blot the microplate on absorbent tissue to remove any excess liquid from the wells.

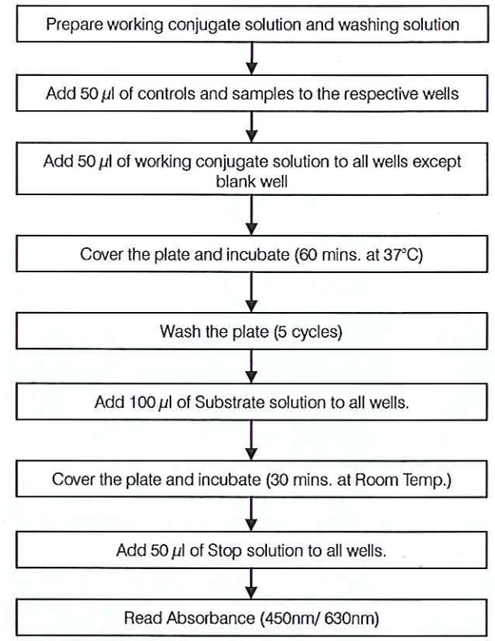
Step 7: Immediately after washing the plate, **add** 100 µl of **Substrate Solution** to each well.

Step 8: Cover the wells with adhesive strip(s) and **incubate** for **30 mins** at room temperature. Keep away from direct sunlight. A Blue or bluish green colour should develop in wells containing reactive samples.

Step 9: Add 50 µl of **Stop Solution** to each well.

Step 10: Within 15 minutes **read** the absorbance at 450 nm using 630 nm as the reference wavelength.

PROCEDURAL FLOW CHART



RESULTS

CALCULATION OF RESULTS

Each plate must be considered separately when calculating and interpreting results of the assay.

Approved software may be used for calculation and interpretation of results.

Negative Control

Calculate the mean absorbance of the Negative Controls.

Example:

Well 1 = 0.009, Well 2 = 0.010, Well 3 = 0.011

Total = 0.030

Mean Negative Control = 0.030/3 = 0.010