MaxLISA HBsAg 3.0 (Ag) ELISA Test

(Enzyme Linked Immunosorbent Assay)

ORDERING INFORMATION

Ref. No.	Pack Size
AVELHBV3-96	96 Tests

1. INTENDED USE

MaxLISA HBsAg 3.0 (Ag) ELISA Test is a one-step enzyme immunoassay diagnostic kit for in vitro qualitative determination of hepatitis B surface antigen (HBsAg) concentration in human serum or plasma (Heparin, Citrate or EDTA). This test is not automated.

2. INTRODUCTION

Hepatitis B (HB) is an infectious disease caused by the Hepatitis B virus (HBV) that affects the liver. It can cause both acute and chronic infections. Transmission of hepatitis B virus results from exposure to infectious blood or body fluids containing blood. It is 50-100 times more infectious than human immunodeficiency virus (HIV). Possible forms of transmission include sexual contact, blood transfusions and transfusion with other human blood products, re use of contaminated needles and syringes, vertical transmission from mother to child during childbirth. The virus may be detected within 30 to 60 days after infection and can persist and develop into chronic hepatitis B. The tests for detection of hepatitis B surface antigen (HBsAg) is most frequently used to screen for the presence of this infection. MaxLISA HBsAg 3.0 (Ag) ELISA Test is a fast test for the qualitative detection of the presence of HBsAg in serum or plasma (heparin, citrate or EDTA) specimen. HBsAg assays are used not only for diagnose HBV infections but also to monitor the course of the disease and the efficacy of antiviral therapy.

3. PRINCIPLE

The MaxLISA HBsAg 3.0 (Ag) ELISA Test is an enzyme-linked immunosorbent assay (ELISA) that directly detects the presence of HBsAg (Hepatitis B surface antigen). The assay utilizes solid-phase technology, where the microwells are coated with monoclonal anti-HBsAg antibodies. The Conjugate used in this test consists of polyclonal anti-HBsAg antibodies labeled with horseradish peroxidase.
During the test, samples and controls are added to the microwells and incubated. If HBsAg antigens are present in the sample, they will bind to the monoclonal anti-HBsAg antibodies immobilized on the microwell surface. Any unbound sample and antigens are then washed away. In the subsequent step, the conjugate is added, and it binds to any specific antigen that is already bound to the antibody on the well. After washing away any unbound conjugate, a substrate solution containing 3, 3, 5, 5'-Tetramethylbenzidine (TMB) and hydrogen peroxide is added to the wells. The wells that have bound conjugate develop a blue to bluish-green color, which is then converted to a yellow to orange color when the reaction is stopped with a stop solution containing sulfuric acid.
The enzyme-substrate reaction is then measured spectrophotometrically. The intensity of the color produced in the wells is directly proportional to the concentration of HBsAg present in the sample. Wells that contain negative samples remain colorless.

4. KIT CONTENTS:

Store all components at 2-8°C when not in use

Material	96 Tests
Microwells (1 x 96 well microplate)	Microwells coated with Anti-HBsAg (monoclonal antibody) and packed in a pouch with desiccant
Conjugate Diluent (1 x 15 ml)	Buffer contains stabilizers
Enzyme Conjugate Concentrate (50X) (1 x 0.3 ml)	Anti HBsAg - Antibody peroxidase conjugate
Wash Buffer (20X) (1 x 25 ml)	Concentrate Phosphate buffer with surfactant
TMB Substrate A (1 x 10 ml)	To be diluted with TMB diluent before use
TMB Substrate B (1 x 10 ml)	Buffer solution containing H_2O_2
Positive Control (1 x 1 ml)	Ready to use, Inactivated and reactive for (1 HBsAg and non-reactive for HIV &HCV
Negative Control (1 x 1 ml)	Ready to use, normal human serum Negative for HBsAg, HCV &HIV
Stop Solution (1 x 12 ml)	Ready to use.
Pack insert	1 No.

5. MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or Deionized water.
- Micropipettes and Micro tips.
- Graduated cylinders for reagent bottles.
- Paper towels or Absorbent tissue.
- 70% Isopropanol solution.
- Vortex mixer.
- Incubator (37°C).
- ELISA Washer.
- ELISA Reader.
- Timer.
- Biohazard waste container with sodium hypochlorite solution.
- Disposable gloves

6. SAMPLE COLLECTION, STORAGE & HANDLING

- Only human serum or plasma samples should be used for the test
- While preparing serum samples, remove the serum from the clot as soon as possible to avoid hemolysis
- Fresh serum/plasma samples are preferred.
- Serum and plasma (EDTA) samples may be stored for up to 7 days at 2-8°C or at least 6 months as frozen (-20 to -70°C)
- Avoid repeated freezing and thawing.
- Do not use sodium azide as preservative because it inactivates horseradish peroxidase
- Microbial contaminated and hemolyzed samples may give erroneous Results.

7 . PRECAUTIONS:

- For in vitro diagnostic use only
- Bring all reagents and specimen to room temperature before use.
- The use of disposable gloves and proper biohazards clothing is strongly recommended while running the test.
- Before performing the test, read all the instructions carefully and follow each and every instruction to get the intended and accurate results.
- Do not eat, drink or smoke in the area where testing is done
- In case there is a cut/wound in hand, do not perform the test.
- Do not pipette any material by mouth.
- Do not mix components of one kit with another.
- Do not allow liquid from one well to mix with other wells.
- Use the required volume of specimen while testing.
- Do not let the strips dry in between the steps.
- Follow GLP and biosafety guidelines for handling and disposal of potentially infective materials/expired kit/used kits.
- Carefully read and follow the assay procedure and storage instructions. Deviation will lead to erroneous results.
- All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121°C for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
- Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact wit heyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
- Stop solution contains sulfuric acid. If sulfuric acid comes in contact with the skin, wash thoroughly with water. In case of contact with eyes, flush with excess of water.
- In case of performance changes or product malfunction, stop using the kit immediately and contact your local distributor.
- Controls and samples to be tested should be handled as potentially hazardous as they are capable of transmitting infection.

8. PREPARATION OF REAGENTS

Note: Before use, allow reagents and samples at room temperature (20-30°C).

8.1. Ready for use reagents:
8.1.1. Microplate:
Each frame support containing 12 strips is wrapped in a sealed foil bag. Cut the bag using scissors or a scalpel above the sealing. Open the bag and take out the frame. Put the unused strips back into the bag. Close the bag carefully and put it back into storage at ± 2-8°C

Caution: Handle Microwell strips with care. Do not touch the bottom exterior surface of the walls.
8.1.2. Negative control
8.1.3. HBsAg positive control
8.1.4. Diluent
8.1.5. Stop solution

8.2. Reagents to reconstitute:
8.2.1. Wash buffer (20X):

8.2.1. Wash buffer (20X):

- Check the buffer concentrate for the presence of salt crystals. If crystals are present in the solution, resolubilize by warming at 37°C until all crystals Dissolve.
- Dilute 1:20 in distilled water to obtain the ready to use washing solution. Mix 25 ml of 25X wash buffer concentrate with 475 ml of distilled or deionized water. Working wash buffer is stable for 2 months when stored at 2-8°C.

8.2.2. Wash Procedure:

- Incomplete washing will adversely affect the test outcome.
- Aspirate the well contents completely into a waste container.
- Then fill the wells completely with wash buffer avoiding overflow of buffer from one well to another well.
- Aspirate completely and repeat the wash procedure for a total 5 times of Washes.
- Automated washer if used should be well adjusted to fill each well Completely.
- Tap plate on absorbent papers till no droplets appear on the paper.

8.2.3. Preparation of working conjugate:

Make a 1:50 dilution of conjugate concentrate with conjugate diluent. Do not store working conjugate. Prepare conjugate 10 minutes before use. Determine the quantity of working conjugate solution to be prepared from table given below.
Mix solution thoroughly before use.

Example:

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
Enzyme Conjugate Concentrate (µl)	20	40	60	80	100	120	140	160	180	200	220	240
Conjugate Diluent in (ml)	1	2	3	4	5	6	7	8	9	10	11	12

8.2.4. Preparation of Substrate:

Mix TMB Substrate A and TMB Substrate B in 1:1 ratio to prepare working substrate buffer 5 to 10 minutes before use. Avoid exposure to light.

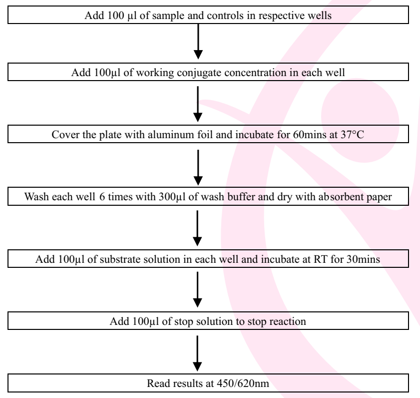
Example:

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
TMB Substrate A (ml)	0.5	1	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
TMB Substrate B (ml)	0.5	1	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0

9. TEST PROCEDURE:

I. Bring all the reagents and specimen to room temperature before use.
II. Take the required number of strips and fix them to frame and immediately close the Pouch.
III. Prepare template in data sheet indicating the location of controls and Specimens.
IV. Add 100µl of negative control in each well no. A1 & B1
V. Add 100 µl positive control in C1 .
VI. Add 100µl of specimen in each well starting from D1.
VII. Add 100µl of working enzyme conjugate to each well.
VIII. Cover the plate with aluminum foil and incubate for 60 minutes at 37°C.
IX. After incubation, aspirate the contents from all the wells and wash each well 6 Times with by filling approximately 300µl diluted wash buffer.
X. Invert the plate and tap it on absorbent paper to remove the remaining washing solution, and then pipette 100µl of prepared diluted substrate into each well and incubate at room temperature for 30 minutes.
XI. Add 100µl of stop solution each well.
XII. Read absorbance at 450nm by keeping 620 nm as a reference wavelength within 30 minutes in ELISA READER

10. FLOW CHART FOR TEST RUN CRITERIA:



11. CALCULATION OF THE CUT-OFF VALUE:

A. Negative control means (Ncx)

A. **Negative control means (NCx)**

	Absorbance of Negative control (NC)
NC1	0.090
NC2	0.082
NC Mean (NCx)	**(0.090+0.082)/2 = 0.086**

B. Cut off value: NCx+0.2 = 0.090 + 0.2 = 0.286

C. Quality Control:
Results of an assay are valid if the following criteria for the controls are met:
PC must be ≥ 0.500.
NC must be ≤ 0.200

D. Abbreviations:
NC - Absorbance of Negative control
Ncx - Mean Negative control
PC - Absorbance of the Positive control

12. INTERPRETATION OF RESULTS:

- Test specimens with absorbance value less than the cut off value are non-reactive and may be considered as negative for HBsAg.
- Test specimens with absorbance value greater than or equal to the cut off value are reactive for HBsAg by HBsAg ELISA.
- Specimens with absorbance value equal to or greater than the cut off value are considered initially reactive by the criteria of HBsAg. If the samples are considered positive, the test should be conducted two or more times.
- If any one of positive duplicates retest sample absorbance value is equal to or greater than the cut off or both duplicate retest values are equal to or greater than the cut off, the specimen is considered reactive by the criteria of HBsAg. Further confirmation by other confirmation assays including western blot or PCR is recommended.

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13. LIMITATIONS:

- The test should be used for detection of HBsAg ins serum or plasma only and not in other body fluids.
- HBsAg is a screening assay, it's not be used as a sole criterion for diagnosis of HBV infection.
- Reactive sample should be retested with confirmatory assays like neutralization assays, HBV DNA by PCR.
- The most common assay mistakes are: using kits beyond the expiry date, wrong washing procedures, contaminated reagents, incorrect assay procedure steps, failure to add specimens or reagents, timing errors, the use of incompletely clotted serum specimens.
- This assay cannot be utilized to test pooled (mixed) plasma. HBsAg has been evaluated only with individual serum or plasma specimens.
- HBsAg is a qualitative assay and the results cannot be used to measure antigen concentration.

14. PERFORMANCE CHARACTERISTICS

Sensitivity

The performance of 3rd Generation MaxLISA HBsAg Test is evaluated in-house with fresh as well as frozen sample from low risk to high risk groups by using a panel containing 905 nos. samples (150 Positive and 755 Negative). Compare with other commercially available ELISA kits. The sample from random blood donors, patients with diseases related to HBsAg and patients with diseases unrelated to HBsAg. The result of all the sera with a difined HBsAg status were fully comparable with those of 3rd Generation MaxLISA HBsAg Test. The result of in-house study are as below table.

HBsAg Reference Method

Type of Specimen	Observation				Total Results
	MaxLISA HBsAg Test		Commercial available HBsAg ELISA		
	Positive	Negative	Positive	Negative	
Positive	150	0	150	0	150
Negative	01	754	0	755	755

on the basic of above Internal Evalutions:
Sensitivity: 100% Specificity: 99.8%

PRECISION

Intra-Assay

Within-run precision has been determined by using 10 replicates of three specimens containing 0 ng/ ml, 1 ng/ ml and 5 ng/ ml of HBsAg. The negative and positive valueswere correctly identified 98% of the time.

Inter-Assay

Between-run precision has been determined by using the same three specimens of 0 ng/ml, 1 ng/ ml and 5 ng/ml of HBsAg in 10 independent assays. Three different lots of the MaxLISA HBsAg 3.0 (Ag) ELISA Test has been tested over a 10 Days using negative, low positive and high positive specimens . The specimens were correctly identified 98% of the time.

15. INTERFERING SUBSTANCES

The following potentially interfering substances were added to HBsAg negative and positive specimens.

Acetaminophen:	20 mg/dL	Caffeine:	20 mg/dL
Acetylsalicylic Acid:	20 mg/dL	Gentisic Acid:	20 mg/dL
Ascorbic Acid:	2g/dL	Albumin:	2 g/dL
Creatin:	200 mg/dL	Hemoglobin	1.1 mg/dL
Bilirubin:	1g/dL	Oxalic Acid: 600mg/dL	

None of the substances at the concentration tested interfered in the assay.

16. REFERENCES:

- World Health Organization: Advance in Viral Hepatitis, Technical report series no.602 (1977), Geneva. Voller A., Bidwell D.E. and Barriett A: The enzyme linked immunosorbent assay (ELISA), Dynatech Europe: Borough House, Ru du pre, Guernsey, G.B. (1979).
- NCCLS Document I 17-P (1991). Protection of laboratory workers For instrument biohazards. Vol.1(15).

Symbols Used on Pack

Symbol	Meaning	Symbol	Meaning
REF	Catalogue Number		Warning/Caution
LOT	Batch No.	IVD	In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
Σ	Contains sufflcient no. of test		

Manufactured in India by :
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Plot No.: 338, Sector-2, Industrial Growth Centre, Saha, Ambala, Haryana (INDIA)-133104
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