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23. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. No colour developed at the end of assay	a) Any one reagent has been added in wrong sequence. b) Inactivated conjugate, improper storage c) Microplate inactivated, due to improper storage d) Inactivated substrate, improper storage or preparation e) Omission of any step in test procedure f) Incorrect (low) incubator temperature, timing or pipetting g) Improper preparation of enzyme conjugate (dilution error) improper mixing of reagents. h) Kit expired/ deteriorated/ reagent of different kit used.	Follow the procedure meticulously & repeat assay. Check storage of enzyme conjugate and it should be free of any contamination. Keep unused strips in aluminium poly pouch with the dessicant pouch inside and proerly closed with clamp & rod provided with the kit. Use freshly prepared substrate solution. Recheck procedure, repeat assay Follow the procedure meticulously & repeat assay. Check incubator temperature, procedure & repeat assay Check procedure & repeat assay Check storage and expiry of the kit before use.
2. High O.D. value of Negative control	a) Plate not stopped after 30 minutes of adding stop solution b) Same microtip used for Positive and negative controls c) Nonspecific attachment/ binding of other reagent while addition of next step.	Follow the procedure meticulously & repeat assay. Change micropipette tips while addition of negative/ positive control If plates get scratches/ aberrations during washing, non specific proteins may bind while addition of next step.
3. Too much colour in all wells of the plate (high background)	a) Contaminated substrate b) Contaminated washing solution (1X). Poor quality of water used for diluting wash buffer conc. c) Over incubation of substrate and delay in addition of stop solution. d) Insufficient washing. i) Washing not consistent due to blockage of probes ii) Filling volume not sufficient. iii) Insufficient no. of wash cycles. iv) Contaminated wash device	Check substrate (TMB Diluent) it should be colourless. If blue in colour then discard and use clean disposable container. Check the container and quality of water used for dilution. Use of distilled water is preferred. Follow the procedure meticulously. Check wash device and clean probes of manifold. fill the well close to the top. After washing, blot the microwells on absorbent tissue. Follow wash protocol meticulously

PROBLEM	POSSIBLE CAUSE	SOLUTION
4. Poor reproducibility	e) Use of wash solution from other manufacturer. f) Working substrate not protected from light a) Washing problems.	Use only Hepalisa wash solution. Incubate the plate in dark after addition of substrate. Check all 8 ports/ manifold for uniform flow of wash buffer. If there are blockage, clean the ports.
5. False Positive	b) Uncalibrated pipettes or tips not well fitted, improper pipetting/ dispensing. c) Reagent & sera not at room temperature or not well mixed before use. d) Interference in optical pathway due to Air bubbles.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling. Equilibrate reagents to room temperature and mix thoroughly before use Clean or dry the bottom of microwells, check for bubbles and repeat the readings.
6. False Negative/ low O.D. of Positive control & positive sample	a) Beside 3a, b, c, d, e & f incorrect interpretation and calculation of final results b) High incubator temperature, incorrect timing or pipetting c) Use of turbid/ lipaemic or hemolyzed sample. a) Inadequate addition of substrate/conjugate solution. b) Kit expired, reagent of different kit used. c) White particles in working substrate solution, again in fresh tube. d) Uncalibrated pipettes, improper pipetting, carefully without bubbling. e) Deterioration of Enzyme conjugate Conc. f) Stop solution is added before 30 minutes. Reaction terminated before 30 minutes. g) O.D. taken at incorrect wavelength. h) Incorrect (low) incubator temperature, timing or pipetting i) Kit deterioration j) Sample deterioration due to improper storage and / or microbial contamination.	Check the calculation part given in the insert and correctly interpret. Check incubator temperature, procedure & repeat assay. Centrifuge the sample at 5000 rpm for 30 minutes and re-run the test with clear sample. Follow the procedure meticulously & repeat assay. Check the expiry of the kit before use. Discard the substrate and prepare the working substrate Use only calibrated pipettes with well fitted tips & pipette Check storage of Enzyme conjugate Conc. It shall be stored at 2-8°C. Follow the test procedure meticulously. Read O.D. values at 450 nm and 630 nm. Check incubator temperature, procedure & repeat assay. Check storage of kit and it should be stored at 2-8°C. Store the sample at 2-8°C / -20°C as recommended in the specimen collection & handling.

in vitro diagnostic Reagent, not for medicinal use

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R-03

HEPALISA

Microwell ELISA Test for the Detection of Hepatitis B Surface Antigen (HBsAg) in Human Serum/ Plasma

1. INTENDED USE

The Viral Hepatitis caused by Hepatitis B Virus is termed as "Serum Hepatitis". 1-5% infected people act as chronic carriers of HBV Virus. Major part of the chronic carriers secrete hepatitis B surface antigen (HBsAg) into blood and other secretions of the body like saliva and vaginal fluid. These chronic carriers are potentially infectious to other seronegative people.

Hepatitis B Virus belongs to a family of enveloped DNA virus, the Hepadnavirus. Related viruses in this group cause chronic hepatitis.

HBsAg has been accepted as a universal and the most reliable seromarker in case of acute HBV infection due to its appearance almost 2-4 weeks before the ALT level becomes abnormal and 3-5 weeks before the onset of symptoms or jaundice as an early detection system for hepatitis infection. In most cases of HBV infection, the incubation period varies from 40 days to 6 months.

Within HBVs, antigenic diversity is recognised in the surface antigens. HBsAg particles contain common "a" antigen, linked to two sets of mutually exclusive determinants, "d" or "y" and "w" or "r" giving the four main types- adw, adr, ayw and ayr.

2. INTENDED USE

Hepalisa is designed for in-vitro qualitative detection of Hepatitis B surface antigen (HBsAg) in human serum or plasma and is used as a screening test for testing of collected blood prior to transfusion.

3. SEROLOGICAL MARKERS OF CLINICAL SIGNIFICANCE FOR HEPATITIS B VIRUS (HBV)

HBsAg: First detected during the incubation period of 6-8 weeks before the appearance of symptoms. It can appear as early as 2 weeks and generally disappears within 3-4 months after exposure. **It is the most reliable & universal marker of HBV infection.** In the carrier and chronic state it persists more than 6 months.

Anti-HBs: Appearance may take several weeks/months after HBsAg clearance causing a 'window' period. Best indicator of recovery & immunity to HBV. Quantitation allows pre-vaccination screening & follow-up of the post-vaccination response.

HBeAg: Appears within 1 week after HBsAg, lasts 3-6 weeks & disappears before HBsAg clearance. Its presence indicates a highly infectious state, except in pre-core mutants. Persistence > 10 weeks suggests progression to chronic carrier state/hepatitis.

Anti-HBe: It appears before clearance of HBsAg, indicates decreasing infectivity and is a good prognosis for the resolution of infection.

IgM anti-HBc: Appears prior to symptoms, found in high titre for a short time during the acute disease stage that covers the serological 'window' period, declines to low levels during recovery. A marker of recent infection. It differentiates between acute & chronic hepatitis.

Anti-HBc total: Appears 4-10 weeks after HBsAg appearance, persists for years/for life, therefore it is a prominent marker of HBV exposure. It is the only indicator of infection in the window period.

HBV-DNA: Detection of low levels by PCR, allows diagnosis of acute/chronic infection/carrier state & also monitors the response to interferon treatment.

All the above antigens and viral DNA polymerase form useful diagnostic markers for HBV.

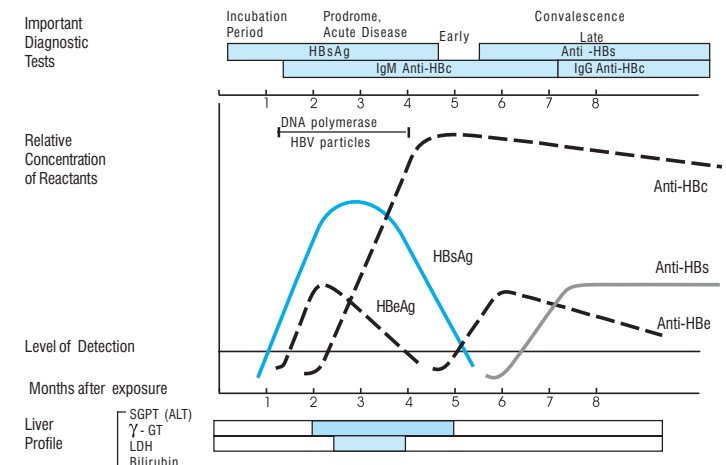


Fig.2: Serological & Clinical pattern during acute HBV infection.

4. PRINCIPLE

HEPALISA is a solid phase enzyme linked immunosorbent assay (ELISA) based on the "Direct Sandwich" principle. The microwells are coated with Monoclonal antibodies with high reactivity for HBsAg. The samples are added in the wells followed by addition of enzyme conjugate (polyclonal antibodies linked to Horseradish Peroxidase (HRPO)). A sandwich complex is formed in the well wherein HBsAg (from serum sample) is "trapped" or "sandwiched" between the antibody and antibody HRPO conjugate. Unbound conjugate is then washed off with wash buffer. The amount of bound peroxidase is proportional to the concentration of HBsAg present in the sample. Upon addition of the substrate buffer and chromogen, a blue colour develops. The intensity of developed blue colour is proportional to the concentration of HBsAg in sample. To limit the enzyme-substrate reaction, stop solution is added and a yellow colour develops which is finally read at 450nm spectrophotometrically.

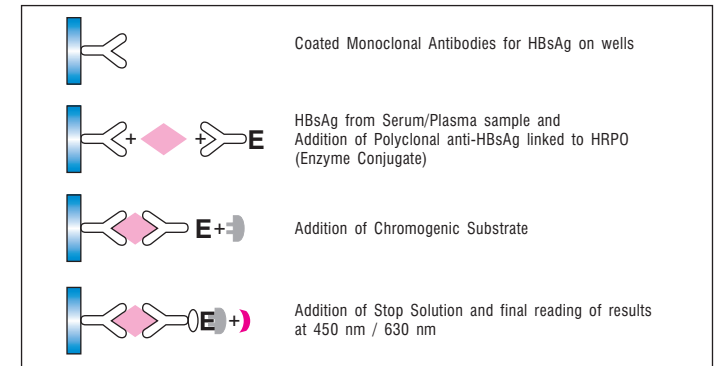


Fig. 3 : Schematic representation of "Hepalisa"

5. DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in European Standard EN ISO 15223-1:2021.

	Manufactured By		In vitro diagnostic medical device
	No. of tests		Instruction for use
	Lot Number Batch Number		Temperature Limitation
	Manufacturing Date		Caution, see instruction for use
	Expiry Date		Catalogue Number
	Keep away from sunlight		Do not use if package is damaged
	Contains biological Material of Human Origin		Contains biological Material of Animal Origin
	Country of Manufacture		Keep Dry

6. KIT PRESENTATION

- 96 Tests

7. KIT & ITS COMPONENTS

PARTICULAR	DESCRIPTION
Microwells	Microwells coated with anti-HBsAg (monoclonal) packed in a pouch provided with dessicant.
Enzyme Conjugate Concentrate (100x)	Containing Polyclonal IgG anti-HBsAg linked to horseradish peroxidase with protein stabilizers.
Conjugate Diluent	Buffer containing protein stabilizers.
Wash Buffer Concentrate (25x)	Concentrated Phosphate buffer with surfactant.
TMB Substrate	To be diluted with TMB diluent before use.
TMB Diluent	Buffer solution containing H ₂ O ₂ with preservative
Control -	Ready to use, normal human serum negative for HBsAg, HCV & HIV.
Control +	Ready to use, inactivated and diluted human serum, reactive for HBsAg and non-reactive for HIV & HCV.
Stop Solution	Ready to use, 1N sulfuric acid.
Plate Sealers	Adhesive backed sheets for sealing microwell plate/strips.

8. STORAGE AND STABILITY

Store all components at 2-8°C when not in use. Expiry date on the kit indicates the date beyond which the kit should not be used.

9. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

- Micropipettes and microtips.
- Elisa reader
- Distilled or deionized water
- Graduated Cylinders, for reagent dilution
- Paper towels or absorbent tissue
- Timer
- Elisa washer
- Incubator 37°C
- Disposable gloves
- Disinfectant Solution

10. SPECIMEN COLLECTION & PREPARATION

- Only human serum 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 samples are preferred.

- Specimens should be free of microbial contamination and may be stored at 2-8°C for one week, or frozen at -20°C or lower. Avoid repeated freezing and thawing.
- Do not use heat inactivated samples as their use may give false results. Haemolyzed and licteric hyperlipemic samples may give erroneous results.
- Do not use Sodium Azide as preservative because it inactivates Horseradish peroxidase.

11. WARNING & PRECAUTION

CAUTION: THIS KIT CONTAINS MATERIALS OF HUMAN ORIGIN. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION. NEGATIVE CONTROL, POSITIVE CONTROL & ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION.

- The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
- In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
- Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
- Tests are for in vitro diagnostic use only and should be run by competent person only.
- Do not pipette by mouth.
- 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 at 15psi 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 with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
- Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
- 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.
- Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
- All materials used in the assay and samples should be disposed off in the manner that will inactivate virus.
- ELISA Reader & micropipettes used in testing should be calibrated at regular interval to ensure accurate results.

12. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

HEPALISA test is best used with fresh samples that have not been frozen and thawed. However most frozen samples will perform well if the procedure suggested below is followed.

Allow the frozen sample to thaw in a vertical position in the rack. Do not shake the sample. This allows particles to settle to the bottom. If a centrifuge is available, the sample should be centrifuged. (10,000 rpm for 15 min.)

(B) TRANSPORTATION

If the specimen is to be transported, it should be packed in compliance with the current Government regulations regarding transport of aetiologic agents.

13. PRECAUTIONS FOR USE

Optimal assay performance requires strict adherence to the assay procedure described in the manual.

- Do not use kit components beyond the expiration date which is printed on the kit.
- Bring all the reagents & samples to room temperature (20-30°C) before use.
- Do not combine reagents from different batches, as they are optimised for individual batch to give best results.
- Avoid microbial contamination of reagents. The use of sterile disposable tips is recommended while removing aliquots from reagent bottles.
- Due to interchange of caps the reagents may get contaminated. Care should be taken while handling the reagents to avoid contamination of any sort.
- Use freshly collected, clean serum samples for assay. Try to avoid turbid, lipemic serum or plasma samples.
- Use a separate tip for each sample and then discard it as biohazardous waste.
- All pipetting steps should be performed with utmost care and accuracy. Cross contamination between reagents and samples will invalidate results.
- Do not allow microwells to dry once the assay has started.
- Run negative and positive controls in each assay to evaluate validity of the kit.
- Incubation time should not vary by more than + 2 min.
- Prevent evaporation during sample incubation by covering the strips with strip sealer. Remove sealer before washing.
- Distilled or deionised water must be used for wash buffer preparation.
- Thorough washing of the wells is critical to the performance of the assay. Overflowing of reagents or washing to adjacent wells must be prevented during washing, which may lead to incorrect results due to carry over effect.
- Take care while preparing working substrate solution and use separate tips for TMB Substrate

and TMB diluent.

- Prepare working substrate solution just 10 minutes prior to adding in the wells.
- If blue colour or white particles appear in working substrate solution then do not use it. Take fresh containers and tips and prepare it again.
- Avoid strong light exposure during the assay.
- Ensure that the microwell strips are levelled in the strip holder. Before reading, wipe the bottom of the microwell strips carefully with soft, absorbent tissue to remove any moisture.
- If available, a microwell reader which contains a reference filter with settings at 620 or 630 nm should be used. Use of a reference filter minimises interference due to microwells that are opaque, scratched or irregular. However, if a reference filter is unavailable, the absorbance may be read at 450 nm without a reference filter.
- In case of any doubt the run should be repeated.

14. PREPARATION OF REAGENTS

Prepare the following reagents before or during assay procedures. Reagents and samples should be at room temperature (20-30°C) before beginning the assay and can remain at room temperature during testing. Return reagents to 2-8°C after use. All containers used for preparation of reagents must be cleaned thoroughly and rinsed with distilled or deionized water. Pre-warm the incubator at 37°C.

14.1. Hepalisa strips:

Bring foil pack to room temperature (20-30°C) before opening to prevent condensation on the microwell strips.

- Break-off the required number of strips needed for the assay and place in the strip holder. Take the strip holder with the required number of strips, taking into account that, two negative and two positive control should be included in the run while opening the fresh kit. However for one or two strips one negative and one positive control and for more strips at least two negative and two positive controls should be included in each subsequent runs.

b. Unused wells should be stored at 2-8°C, with dessicant in a aluminium pouch with clamp & rod. Microwells are stable for 30 days at 2-8°C from the date of opening of sealed pouch, when stored with desiccant along with clamp & rod.

Caution: Handle microwell strip with care. Do not touch the bottom exterior surface of the wells.

14.2. Preparation of Wash Buffer:

- 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.

- Prepare at least 25ml. (1ml. concentrated wash buffer with 24 ml. water) of working wash buffer for each strip used. Mix well before use.

- Mix 20 ml. of 25X wash buffer concentrate with 480ml. of distilled or deionized water. **Working Wash Buffer is stable for 2 months when stored at 2-8°C.**

14.3. Preparation of Working Conjugate:

Dilute conjugate concentrate 1:50 in conjugate diluent. Do not store working conjugate. Prepare a fresh dilution for each assay in a clean glass vessel. Determine the quantity of working conjugate solution to be prepared from table given below. Mix solution thoroughly before use.

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)	10	20	30	40	50	60	70	80	90	100	110	120
Conjugate Diluent in (ml)	1	2	3	4	5	6	7	8	9	10	11	12

Note: In case any precipitate is found in conjugate diluent, it should be allowed to settle and the supernatant can be used for the test. The precipitate does not interfere with the working of the kit.

14.4. Preparation of working substrate solution :

Mix TMB substrate and TMB Diluent in 1:1 ratio to prepare working substrate.

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 (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
TMB Diluent (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0

Do not store working substrate. Prepare a fresh dilution for each assay in a clean plastic/glass vessel. Determine the quantity of working substrate solution to be prepared from table. Mix solution thoroughly before use. Discard unused solution. A deep blue color present in the substrate solution indicates that the solution has been contaminated and must be discarded.

15. 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 and allow to soak (approx. 30 seconds). Aspirate completely and repeat the wash and soak procedure 5 additional times for a total of 6 washes.
- Automated washer if used should be well adjusted to fill each well completely without over filling.
- Tap upside down on absorbent sheet till no droplets appear on the sheet, taking care not to dislodge the wells.

16. TEST PROCEDURE

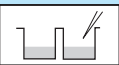

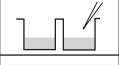

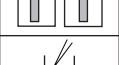
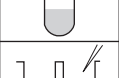

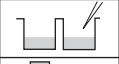

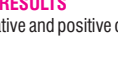
The instructions of the procedure must be strictly followed.

Fit the strip holder with the required number of HEPALISA strips. The sequence of the procedure must be carefully followed. Arrange the assay control wells in a horizontal or vertical configuration. Configuration is dependent upon reader software.

- Add 100 µl Negative Control in each well No. A-1 and B-1 respectively.
- Add 100 µl Positive Control in C-1 & D-1 wells.
- Add 100 µl of sample in each well, starting from E1.
- Add 50 µl of working Enzyme conjugate to each well. Gently shake the plate for 2-3 seconds to mix the sample & conjugate.
- Cover the plate and incubate in an incubator at 37°C + 1°C for 60 minutes.
- Dilute the wash buffer concentrate with distilled water to 1:25 dilution.
- At the end of incubation period, take out the plate from incubator and wash with working wash buffer.

WASHING: Washing can be performed either with ELISA WASHER or manually as follows:

- Empty the wells
- Add 300-350 µl of working washing solution into each well and give a soak time of 30 seconds.
- Empty the wells.
- Wash each well six times in total.
- After the sixth wash, tap dry the Microwells a few times on an absorbent tissue.
- Tap dry the wells after washing and add 100 µl of working substrate solution in all the wells.
- Cover the plate with an aluminium foil and incubate at room temperature (20-25°C) for 30 minutes in dark.
- Stop the reaction by adding 100 µl of stop solution to each well, mix gently.
- Read absorbance at 450 nm. within 30 minutes in ELISA READER. (Bichromatic absorbance measurement with a reference wavelength 600-650 nm is recommended when available).

SUMMARY OF PROCEDURE		
Add controls and samples		100 µl
Prepare working conjugate		No. of Strips 1 2 3 4 5 6 7 8 9 10 11 12 Enz. conc. (µl) 10 20 30 40 50 60 70 80 90 100 110 120 Diluent (ml.) 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0
Add Conjugate		50 µl
Cover the plate & incubate		60 mins. at 37°C
Wash		6 Cycles
Prepare Chromogenic Substrate		No. of Strips 1 2 3 4 5 6 7 8 9 10 11 12 TMB Substrate (ml) 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 TMB Diluent (ml) 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0
Add Substrate		100 µl
Incubate in dark		30 mins. at Room Temp.
Add Stop Solution		100 µl
Read Results		450 nm./630 nm.

17. CALCULATION OF RESULTS

Compute mean of negative and positive control absorbance.

Test Validity :

Positive Control Acceptance Criteria:

PC or PCx must be > 0.5. If it is not so, the run is invalid and must be repeated.

PC	1.430	C1 Well
	1.500	D1 Well
Total	2.930	

Mean absorbance, PCx = 2.930/2 = 1.465

Negative Control Acceptance Criteria:

NC must be < 0.150

NC	0.012	A1 Well
	0.010	B1 Well
Total	0.022	

Mean absorbance NCx = 0.022/2 = 0.011

Cut-off Value

Cut-off value can be determined by using the following formula:

Cut-off Value = NCx + 0.1

Where NCx is mean absorbance (O.D) of Negative Control.

e.g. 0.011 + 0.1 = 0.111

18. INTERPRETATION OF RESULTS

The absorbance of the unknown sample is compared with the calculated cut-off value.

- Test specimens with absorbance (O.D.) value less than cut-off value are non reactive and may be considered as negative for HBsAg.
- Test specimens with absorbance (O.D.) value greater than or equal to cut off value are reactive for HBsAg by HEPALISA.
- Test specimens with absorbance value within 10% below the cut off should be considered suspect for the presence of HBsAg and should be retested in duplicate.
- Specimens with absorbance value equal to or greater than the cut off value are considered initially reactive by the criteria of Hepalisa. Original specimen should be retested in duplicate.
- If both duplicate retest sample absorbance value is less than cutoff value, the specimen is considered non-reactive.
- If any one of the duplicates retest sample absorbance value is equal to or greater than the cutoff or both duplicate retest value are equal to or greater than the cutoff, the specimen is considered reactive by the criteria of HEPALISA. Further confirmation by other EIA assays or confirmatory assays are recommended.

19. PERFORMANCE CHARACTERISTICS

- Analytical Sensitivity : The sensitivity of the kit has been determined using WHO standard (Second international standard for HBsAg subype adw2 geno type A), NIBSC Code No.: 00/588 and it is equal to 0.250 IU/ml.

Hepalisa has a sensitivity of 0.1 ng/ml for all the 11 subtypes (ad & ay subtype) including mutant strains.

- Subtype specificity of Hepalisa has been tested with the HBsAg subtype panel consisting of the following HBsAg subtypes: ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrq+adyw, adr(q neg) and ayw3. (intermediate between ayw 3 and ayw 4) . All of these subtypes were clearly HBsAg reactive with Hepalisa.

- Performance of Hepalisa with reference to sensitivity and specificity has been determined by W.H.O., Geneva. The samples included in the panels for evaluation were from Latin American, Asian, European and African origin. The panels also included various sero conversion panels from Boston Biomedica Inc. (BBI), world wide performance panel and anti-HBsAg low titre performance panel. The evaluation indicate the following sensitivity and specificity:

Sensitivity: 100%

Specificity: 100%

As per WHO Evaluation Report 2004-1 Hepatitis B Surface Antigen Assays: Operational Characteristics (Phase 1) Report-2.

This information is provided for the Scientific Community Enquiring for an independent evaluation other than company's in house evaluation. It is not for commercial or promotional purpose.

- The performance of Hepalisa has also been evaluated in house with fresh as well as frozen samples from low risk as well as high risk groups by using a panel containing 2813 nos. of known samples. The testing has been done with clinical samples, samples from random blood donors, cross reacting samples; RA, CRP ASO and patients with diseases related to HBV. The results of all the samples with a difined HBV status were fully comparable with those of Hepalisa. The results of in-house studies are as follows:

No. of	Status	Hepalisa + ve	Hepalisa - ve
305	All ELISA +ve	305	-
2508	EIA -ve	1	2507

SENSITIVITY: 100 %

SPECIFICITY: 99.92%

Precision: Within-run and between-run precisions have been determined by testing 10 replicates of eight specimens: three negative, five HBsAg positive sample(two weak, two medium and a strong positive). The C.V. (%) of all eight samples were within 10%.

20. LIMITATION OF THE TEST

- The test should be used for detection of HBsAg in serum or plasma only and not in other body fluids.
- This is only a screening test and will only indicate the presence or absence of Hepatitis B Surface Antigen in the specimen. All reactive samples should be confirmed by confirmatory test. Therefore for a definitive diagnosis, the patients clinical history, symptomatology as well as serological data should be considered. The results should be reported only after complying with the above procedure.
- False positive results can be obtained due to the presence of Rf antibodies, patients with auto-immune disease, liver problems, renal disorders and antenatal samples.

21. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in-vitro diagnostic assay within the limitations and specifications as described in the product instruction for use, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacture's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed.

The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

22. REFERENCES

- A.BONILO, M. DOVIS, R. MATTEJA

The use of an enzyme linked immunosorbent assay for screening hybridoma antibodies against Hepatitis B Surface antigen. J. Immunol. Meth, 49:1 (1982)