HEPALISA

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

1. INTRODUCTION

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 than6 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.

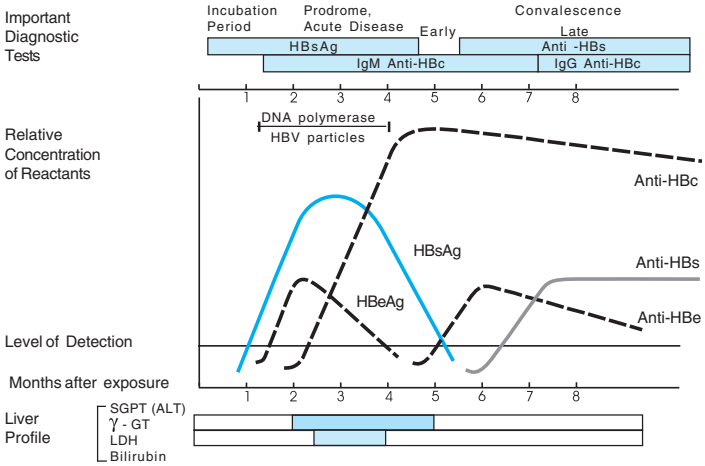

Fig.2: Serological & Clinical pattern during acute HBV infection.

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.

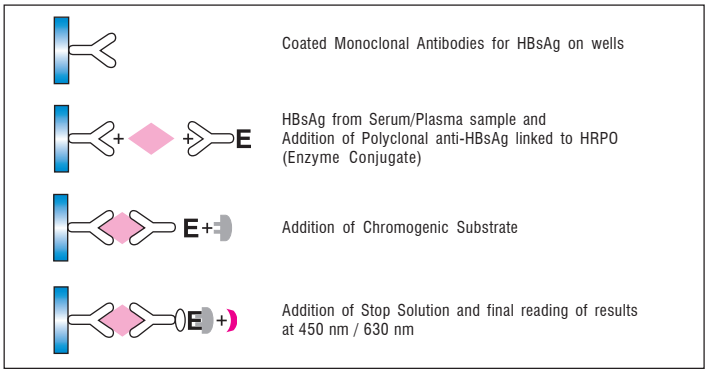

Fig. 3 : Schematic representation of "Hepalisa"

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 the European Standard EN ISO 15223-1:2016.

	Manufactured By	IVD	*In vitro* diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number Batch Number	2°C 8°C	Temperature Limitation
	Manufacturing Date		Caution See instruction for use
	Expiry Date	REF	Catalogue Number
	Do not use if package is damaged		Keep away from sunlight

6. KIT PRESENTATION

96 Test Pack

7. KIT & ITS COMPONENTS

PARTICULAR	DESCRIPTION	QUANTITY
Microwells	**Microwells** coated with anti-HBsAg (monoclonal) packed in a pouch provided with desiccant.	1 Plate (12 × 8 wells)
Enzyme Conjugate Concentrate (50X)	Containing Polyclonal IgG anti-HBsAg linked to horseradish peroxidase with protein stabilizers	1 Vial (0.25 ml)
Conjugate Diluent	Buffer containing stabilizers.	1 Bottle (9 ml)
Wash Buffer Concentrate (25X)	Concentrated Phosphate buffer with surfactant.	1 Bottle (35 ml)
TMB Substrate	To be diluted with TMB diluent before use.	1 Bottle (10 ml)
TMB Diluent	Buffer solution containing H_2O_2 with preservative	1 Bottle (10 ml)
Control -	Ready to use, normal human serum negative for HBsAg, HCV & HIV .	1 Vial (2.0 ml)
Control +	Ready to use, inactivated and diluted human serum, reactive for HBsAg and non-reactive for HIV & HCV.	1 Vial (2.0 ml)
Stop Solution	Ready to use, 1N H_2SO_4.	1 Vial (15 ml)
Plate Sealers	Adhesive sheets to cover the microwells during incubation.	4 Nos

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 components should not be used.

9. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

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

10. SPECIMEN COLLECTION & HANDLING

1. 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.
2. 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.
3. Do not use heat inactivated samples as their use may give false results. Hemolyzed and Icteric hyperlipemic samples may give erroneous results.
4. **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.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for *in vitro* diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. 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.
7. 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.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. 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.
10. All materials used in the assay and samples should be disposed off in the manner that will inactivate virus.
11. 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.

1. Do not use kit components beyond the expiration date which is printed on the kit.
2. Bring all the reagents & samples to room temperature (20-30°C) before use.
3. Do not combine reagents from different batches, as they are optimised for individual batch to give best results.
4. Avoid microbial contamination of reagents. The use of sterile disposable tips is recommended while removing aliquots from reagent bottles.
5. Due to interchange of caps the reagents may get contaminated. Care should be taken while handling the reagents to avoid contamination of any sort.
6. Use freshly collected, clean serum samples for assay. Try to avoid turbid, lipemic serum or plasma samples.
7. Use a separate tip for each sample and then discard it as biohazardous waste.
8. All pipetting steps should be performed with utmost care and accuracy. Cross contamination between reagents and samples will invalidate results.
9. Do not allow microwells to dry once the assay has started.
10. Run negative and positive controls in each assay to evaluate validity of the kit.
11. Incubation time should not vary by more than ± 2 min.
12. Prevent evaporation during sample incubation by covering the strips with strip sealer. Remove sealer before washing.
13. Distilled or deionised water must be used for wash buffer preparation.
14. 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.
15. Take care while preparing working substrate solution and use separate tips for TMB Substrate and TMB diluent.
16. Prepare working substrate solution just 10 minutes prior to adding in the wells.
17. 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.
18. Avoid strong light exposure during the assay.
19. 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.
20. 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.
21. In case of any doubt the run should be repeated.

14. PREPARATION OF REAGENTS

- Pre-warm the incubator to + 37°C.
- Bring foil pack to room temperature (20-25°C) before opening to prevent condensation on the microwell strips.

a. 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 an 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 desicant along with clamp & rod.

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

- **Preparation of Working Wash Buffer:**

a) Check the wash buffer concentrate for the presence of salt crystals. If crystals are present in the solution, wash resolubilize by warming at 37°C until all crystals dissolve.

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

c) 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.

- **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)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0

Note: In case any precipitate is found in conjugate diluent/sample 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.

- **Preparation of working substrate solution :**

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

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.

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 Susbstrate (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

Discard unused working substrate solution. A deep blue color present in the substrate solution indicates that the solution has been contaminated and must be discarded.

15. WASH PROCEDURE

1. Incomplete washing will adversely affect the test outcome.
2. 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.
3. Automated washer if used should be well adjusted to fill each well completely without over filling
4. 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 stripholder 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.

1. Add 100 μl Negative Control in each well No. A-1 and B-1 respectively.
2. Add 100 μl Positive Control in C-1 & D-1 wells.
3. Add 100 μl of sample in each well, starting from E1.
4. Add 50μl of working Enzyme conjugate to each well. Gently shake the plate for 2-3 seconds to mix the sample & conjugate.
5. Cover the plate and incubate in an incubator at 37°C $\pm$ 1°C for 60 minutes.
6. Dilute the wash buffer concentrate with distilled water to 1:25 dilution.
7. 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:

a). Empty the wells

b). Add 300-350μl of working washing solution into each well and give a soak time of 30 seconds.

c). Empty the wells.

d). Wash each well six times in total.

e). After the sixth wash, tap dry the Microwells a few times on an absorbent tissue.

8. Tap dry the wells after washing and add 100μl of working substrate solution in all the wells.
9. Cover the plate with an aluminium foil and incubate at room temperature (20-25°C) for 30 minutes in dark.
10. Stop the reaction by adding 100μl of stop solution to each well, mix gently.
11. 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 PC$\bar{x}$ must be >0.5. If it is not so, the run is invalid and must be repeated.

	1.430	C1 Well
PC	1.500	D1 Well
Total	2.930	

Mean absorbance, PC$\bar{x}$ = 2.930/2 = 1.465

Negative Control Acceptance Criteria:

NC must be < 0.150

	0.012	A1 Well
NC	0.010	B1 Well
Total	0.022	

Mean absorbance **NC$\bar{x}$** = 0.022/2 = 0.011

Cut-off Value

Cut-off value can be determined by using the following formula:
Cut-off Value = NC$\bar{x}$ + 0.1
Where NC$\bar{x}$ 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.

a) Test specimens with absorbance (O.D.) value less than cut-off value are non reactive and may be considered as negative for HBsAg.
b) Test specimens with absorbance (O.D.) value greater than or equal to cut off value are reactive for HBsAg by HEPALISA.
c) 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.
d) 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.
e) If both duplicate retest sample absorbance value is less than cutoff value, the specimen is considered non-reactive.
f) 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

(i) **Accuracy**: The **Analytical Sensitivity** of Hepalisa is **0.1ng/ml**.

(ii) Subtype specificity of Hepalisa has been tested with the HBsAg subtype panel consisting of the following HBsAg subtypes: *ayw$_1$, ayw$_2$, ayw$_3$, ayw$_4$, ayr, adw$_2$, adw$_4$, adrq+adyw, adr(q* neg) and *ayw3.* (intermediate between ayw *3* and ayw *4)* . All of these subtypes were clearly HBsAg reactive with Hepalisa.

(iii) 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.

(iv) 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 Samples	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

1. The test should be used for detection of HBsAg in serum or plasma only and not in other body fluids.
2. **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.

3. 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-manual, 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.

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23. TROUBLE SHOOTING CHART

	PROBLEM	POSSIBLE CAUSE	SOLUTION
1.	Control out of validation limit	a) Incorrect temperature timing or pipetting	Check procedure & repeat assay
		b) Improper preparation of reagents, error of dilution, improper mixing of reagents.	Check procedure & repeat assay
		c) Cross contamination of Controls	Pipette carefully and do not interchange caps. Repeat assay
		d) Incorrect reading filter or readings without blanking the reader.	Check the filter used. It should be 450nm. If no reference filter is used absorbance will increase.
		e) Interference in the optical pathway	Check the reader. Clean or dry the bottom of micro wells, check for bubbles & repeat the readings.
		f) Used components from different lots.	Do not use components from different lots as they are adjusted for each batch released.
		g) Expired Reagents	Check the kit expiry date. Use the kit with-in shelf life
		h) Use of non calibrated micropipette and/or ELISA Reader	Calibrate micropipette and ELISA Reader at defined interval.
2.	No colour or light colour developed at the end of assay	a) Any one reagent has been added in wrong sequence.	Check procedure and repeat assay.
		b) Inactivated conjugate, wrong dilution used, improper conservation	Check for contamination, recheck procedure
		c) Microplate inactivated, due to improper conservation	Keep unused strips in sealable plastic bag, very well closed with the dessicant pouch inside
		d) Inactivated substrate, improper conservation or preparation	Use freshly prepared substrate solution Recheck procedure, repeat assay
3.	Too much colour in all wells of the plate	a) Contaminated substrate use of same container for preparing & dispensing substrate & conjugate.	Check substrate (TMB Diluent) it should be colourless. If blue in colour then discard and use acid washed or disposable container.
		b) Contaminated or improper dilution of reagents.	Check for contamination, check dilutions.
		c) Contaminated washing solution (1X).	Check the container and quality of water used for dilution.
		d) Over incubation of substrate and delay in addition of stop solution.	Repeat assay.
		e) Insufficient washing.	Check wash device, fill the
		i) Washing not consistent	well close to the top.
		ii) Filling volume not sufficient.	After washing, blot the
		iii) Insufficient no. of wash cycles.	microwells on absorbent tissue.
		iv) Contaminated wash device	
		f) Use of wash solution from other manufacturer.	Use only HEPALISA wash solution.
4.	Poor reproducibility	a) Washing problems.	
		b) Uncalibrated pipettes or tips not well fitted, improper pipetting.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
		c) Reagent & sera not at room temperature or not well mixed before use.	Equilibrate reagents to room temperature and mix thoroughly before use
		d) Too long time for addition of samples or reagents, Inconsistency in time intervals	Develop consistent and uniform technique.
		e) Interference in optical pathway due to Air bubbles.	Refer 1(e).
5.	False Positive	Beside 3a, b, c, d, e incorrect interpretation and calculation of final results	Check the calculation part given in the insert and correctly interpret.
6.	False Negative/ low O.D. for PC and positive sample	a) Sample used was having sodium azide as preservative	Do not add azide in samples
		b Inadequate addition of substrate/conjugate solution	Recheck the test procedure and reagent volume.
		c) Kit expired, reagent of different kit used.	Check the expiry of the kit before use.
		d) White particles in working substrate solution.	Discard the substrate and prepare the working substrate again in fresh tube.
		e) Uncalibrated pipettes, improper pipetting.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.

For *in vitro* diagnostic use only, not for medicinal use

VER-02 R-02

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MN/EBV/015
Rev. Date: Jan.-20