4TH GENERATION

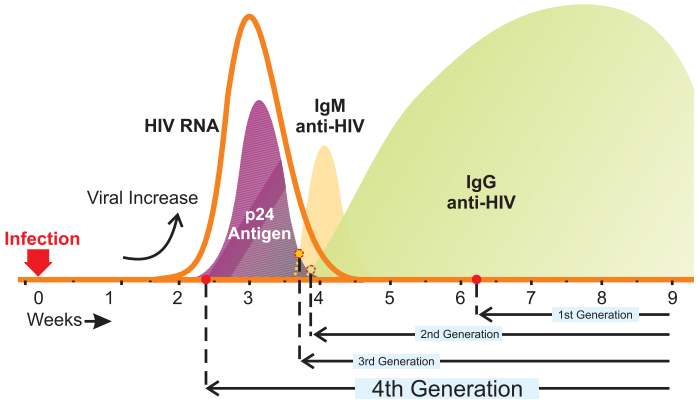
Microlisa - HIV Ag & Ab

Microwell ELISA Test for the Detection of HIV-1 p24 antigen and Antibodies to HIV-1 (Including Subgroup O & C) and HIV-2 in Human Serum/ Plasma

1. SUMMARY AND EXPLANATION OF THE TEST

The available research data indicates that Acquired Immunodeficiency Syndrome (AIDS) is caused by HIV virus and is transmitted by sexual contact, exposure to blood or certain blood products, by an infected mother to her child during pre-natal and post-natal period. The two type of HIV viruses (HIV-1 & HIV-2) have been isolated from patients with AIDS and AIDS related complex (ARC). These two viruses belong to the retrovirus group and are slow viruses.

The serological events following HIV infection are represented graphically in fig.1. In individuals infected with HIV, antigen appears first before anti-HIV but due to seroconversation, the antigen is lost and antibody develops within 1-2 months after infection and thereby the level of the antibody increases.



MICROLISA HIV (Ag & Ab) is developed to detect anti-HIV ENV (envelope) antibodies to HIV-1 and / or HIV-2 with equal reactivity and HIV-1 Antigen. Antigen can generally be detected in acute phase and during symptomatic phase of AIDS and antibodies can be detected througout the infection.
It has been observed that the core protein of HIV-1 and HIV-2 show cross reactivity whereas envelope proteins are more type specific and moreover antibodies against the envelope gene products can be found in almost all infected people. Microlisa HIV (Ag & Ab) has been developed and designed to be extremely sensitive and specific using recombinant proteins; gp41, C terminus of gp120 and gp36 representing the immunodominant regions of HIV-1 & HIV-2 envelope gene structure respectively and HIV-1 p24 antibodies.

2. INTENDED USE

Microlisa HIV (Ag & Ab) is an in vitro qualitative enzyme immunoassay for the detection of antibodies to HIV-1 and / or HIV-2 and HIV -1 P24 antigen in human serum or plasma. It is intended for screening of blood donors or other individuals at risk for HIV-1 and / or HIV-2 infection and for clinical diagnostic testing.

3. PRINCIPLE OF THE TEST

Microlisa HIV (Ag & Ab) test is an enzyme immunoassay based on "Sandwich ELISA".
HIV envelope proteins gp41, C terminus of gp 120 for HIV-1, and gp 36 for HIV-2 representing immunodominant epitopes and anti-HIV-1 p24 antibodies are coated onto microtiter wells. Specimens and controls are added to the microtiter wells and incubated. Antibodies to HIV-1 and HIV-2 and/or HIV p24 antigen if present in the specimen, will bind to the specific HIV antigens and/or anti-p24 antibodies absorbed onto the surface of the wells. The plate is then washed to remove unbound material. Horseradish peroxidase (HRP) conjugated gp41, C terminus of gp120 of HIV-1 and gp36 of HIV-2 and anti-HIV-1 p24 antibodies is added to each well. This conjugate will bind to HIV antigen-antibody or anti-p24 antibody-antigen complex present. Finally substrate solution containing chromogen and hydrogen peroxide is added to the wells and incubated. A blue colour will develop in proportion to the amount of HIV-1 and / or HIV-2 antibodies and/or HIV-1 p24 antigen present in the specimen. The colour reaction is stopped by a stop solution. The enzyme substrate reaction is read by EIA reader for absorbance at a wavelength of 450 nm. If the sample does not contain HIV-1 or HIV-2 antibodies and/or HIV-1 p24 antigen then enzyme conjugate will not bind and the solution in the wells will be either colourless or only a faint background colour develops.

4. 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
- No. of tests
- *In vitro* diagnostic medical device
- See Instruction for use
- Lot Number / Batch Number
- Manufacturing Date
- Expiry Date
- Do not use if package is damaged
- Keep Dry
- Temperature Limitation
- Caution - See instruction for use
- Catalogue Number
- Keep away from sunlight

5. PACK SIZE

- 96 Tests

6. 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.

7. KIT & ITS COMPONENTS

Microlisa HIV (Ag & Ab) Strip Plates	12 Strips (8 well each) Breakway microwells coated with HIV-1 & HIV-2 recombinant proteins and HIV -1 P24 antibody packed in a pouch with dessicant.
Sample Diluent	1 Bottle (8 ml.) Buffer containing protein stabilizers and antimicrobial agents as preservative.
Enzyme Conjugate Concentrate (100X)	1 Vial (0.25 ml.) HIV-1 & 2 Antigens and P24 antibodies labelled with horseradish peroxidase with protein stabilizers.
Conjugate Diluent	1 Bottle (15 ml.) Buffer containing stabilizers.
Wash Buffer Concentrate (25X)	1 Bottle (50 ml) PBS with surfactant. Dilute 1:25 with distilled water before use.
TMB Substrate	1 Bottle (10 ml) To be diluted in TMB Diluent before use.
TMB Diluent	1 Bottle (10 ml.) Buffer solution containing H_2O_2 with preservative.
Control —	1 Vial (2 ml.) Ready to use, normal human serum negative for HIV, HCV, and HBsAg.
Control-1 +	1 Vial (2 ml.) Ready to use, inactivated (HIV) &/or anti-p24 antibody human serum, positive for HIV antibodies and non-reactive for HBsAg and HCV antibodies with preservative. Optimized/ standardized for this kit. Do not use it with other HIV kits/tests.
Control-2 +	1 vial (2 ml) Ready to use HIV - 1 p24 Ag Positive control inactivated, with Preservatives.
Stop Solution	1 Vial (15 ml.) Ready to use, 1N sulfuric acid
Plate Sealers	Adhesive backed sheets for sealing microtiter plate/strips

7. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

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

8. SPECIMEN COLLECTION & PREPARATION

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 haemolysis. 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. Use of heat inactivated, icteric hyperlipemic and haemolyzed samples should be avoided as may give erroneous results.

Note : It is recommended to use kit Positive Control-2 and native samples (serum/plasma) only for p-24 testing.

9. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

Microlisa HIV (Ag & Ab) 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 at 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.

10. WARNING & PRECAUTION

⚠ **CAUTION:** NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION. ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED CAREFULLY 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. Controls contain Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976.)
10. 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.
11. ELISA Reader & micropipettes used in testing should be calibrated at regular interval to ensure accurate results.

11. 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. Prepare working substrate solution just 10 minutes prior to adding in the wells.
16. 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.
17. Use separate tips for TMB substrate and TMB diluent.
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.

12. 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. Prewarm the incubator to 37°C.

1. Microlisa HIV (Ag & Ab) Strip:

Bring foil pack to room temperature (20-30°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 well holder. Take the strip holder with the required number of strips, taking into account that three negative & one positive control-1 and one positive control-2 should be included in the run while opening the fresh kit. However for one or two strips, two negative and one positive control-1 and for more strips at least three negative and one positive control-1 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.

2. Preparation of Wash Buffer:

a) 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.

b) Prepare at least 50 ml. (2ml. concentrated buffer with 48 ml. water) of buffer for each microlisa strip used. Mix well before use.

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

3. Preparation of Working Conjugate:

Dilute conjugate concentrate 1:100 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/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.

4. 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

13. 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 absorbant sheet till no droplets appear on the sheet, taking care not to dislodge the wells.

14. TEST PROCEDURE

Once the assay has started, complete the procedure without interruption. All the reagents should be dispensed in the centre of the well and the tip of the pipette should not touch the wall of the microwell.

Fit the stripholder with the required number of Microlisa HIV (Ag & Ab) strips. The sequence of the procedure must be carefully followed. From well A-1, arrange all controls in a horizontal or vertical configuration. Configuration is dependent upon reader software.

1. Add 25 μl sample diluent in each well.
2. Add 100μl Negative Control in each well no. A-1, B-1 & C-1 respectively.
3. Add 100μl PC-1 in D-1 wells and PC-2 in E1wells.
4. Add 100 μl sample in each well starting from F-1.
5. Apply cover seal.
6. Incubate at 37^{0}C $\pm$ 2^{0}C for 60 min. $\pm$ 2 min.
7. While the samples are incubating, prepare Working Wash Solution and Working Conjugate as specified in Preparation of Reagents.
8. Take out the plate form the incubator after the incubation time is over and, wash the wells 6 times with Working Wash solution according to the wash procedure given in the previous section (wash procedure).
9. Add 100 μl of Working Conjugate Solution in each well.
10. Apply cover seal.
11. Incubate at 37^{0}C $\pm$ 2^{0}C for 30 min. $\pm$ 2 min.
12. Aspirate and wash as described in step no. 8.
13. Add 100 μl of working substrate solution in each well.
14. Incubate at room temperature (20 - 30^{0}C) for 30 min. in dark.
15. Add 100 μl of stop solution.
16. 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).

15. SUMMARY OF PROCEDURE

Add Sample Diluent		Sample Diluent 25 μl
Add control & samples		100 μl
Cover the plate & incubate		60 mins. at 37^{o}C
Wash		6 Cycles
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 Conj. Diluent (ml): 1 2 3 4 5 6 7 8 9 10 11 12
Add Conjugate		100 μl
Cover the plate & incubate		30 mins. at 37^{o}C
Wash		6 Cycles
Prepare Chromogenic Substrate		No. of Strips: 1 2 3 4 5 6 7 8 9 10 11 12 TMB Subs. (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.

16. CALCULATION OF RESULTS

Abbreviations

NC - Absorbance of the Negative Control
NC$\bar{x}$ - Mean Negative Control
PC - Absorbance of the Positive Control

TEST VALIDITY:

Negative Control Acceptance Criteria:

NC$\bar{x}$ must be $\leq$ 0.150. If it is not so, the run is invalid and must be repeated.

Positive Control Acceptance Criteria:

1. PC-1 must be $\geq$ 0.50
2. PC-2 must be $\geq$ 0.400

CUT OFF VALUE

Absorbance

NC - 0.041 A1 Well
- 0.042 B1 Well
- 0.040 C1 Well

Total : 0.123 3 Wells

NC$\bar{x}$ = 0.123/3 = 0.041

Cut-off value is determined by using the following formula:

Cut-off Value = NC$\bar{x}$ + 0.20

Where NC$\bar{x}$ is mean absorbance (O.D) of Negative Control.

e.g. 0.041 +0.20 = 0.241

17. INTERPRETATION OF RESULTS

1. Test specimens with absorbance value less than the cut off value are non-reactive and may be considered as negative for anti-HIV and HIV-1 p24 antigen.
2. Test specimens with absorbance value greater than or equal to the cut off value are reactive for anti-HIV and/or HIV-1 p24 antigen by Microlisa HIV (Ag & Ab).

Note: Test specimens with absorbance value within 10% below the cut off should be considered suspect for the presence of antibodies and/or antigen, should be retested in duplicate.

3. Specimens with absorbance value equal to or greater than the cut off value are considered initially reactive by the criteria of Microlisa HIV (Ag & Ab). Original specimen should be retested in duplicate.
4. If both duplicate retest sample absorbance value is less than cut off value, the specimen is considered non reactive.
5. If any one of the duplicates retest sample absorbance value is equal to or greater than the cut off or both duplicate retest value are equal to or greater than the cut off, the specimen is considered reactive by the criteria of Microlisa HIV (Ag & Ab). Further confirmation by other EIA assays or confirmation assays including Western Blot or PCR is recommended.
6. Specimens which are not repeatedly reactive, may have shown colour due to:

a) Carry over of a highly reactive sample due to contamination of pipette tips.
b) Substrate contamination
c) Inadequate wash or aspiration during wash procedure.

18. LIMITATIONS OF THE ASSAY

1. Microlisa HIV (Ag & Ab) assay is designed for testing antibodies against HIV-1 and/or HIV-2 and HIV-1 antigen in human serum and plasma. Other body fluids and pooled samples are not recommended in this assay. Any result derived from the test of any other body fluid or from test of pooled serum/plasma may not be interpreted correctly based on the current criteria. In establishing infection of HIV-1 and/or HIV-2 or, in evaluating patients with AIDS symptoms, Microlisa HIV (Ag & Ab) testing alone cannot be used to diagnose AIDS even if antibodies and/or antigen against HIV are present in human serum or plasma. A negative test result at any time does not preclude the possibility of exposure to, or infection with HIV. **This is only a screening test.** All samples detected reactive must be confirmed by using Western Blot or PCR. Therefore for a definitive diagnosis, the patient's clinical history, symptomatology as well as serological data should be considered. The results should be reported only after complying with the above procedure.

2. Some samples show cross reactivity for HIV antibodies. **Following factors are found to cause false positive HIV antibody test results:** Naturally occurring antibodies, Passive immunization, Leprosy, Renal disorder, Tuberculosis, Mycobacterium avium, Herpes simplex etc.

19. PERFORMANCE CHARACTERISTICS

(i) Analytical Sensitivity: The sensitivity of the kit has been determined for p24 Antigen using WHO international standard: HIV-1 p24 antigen NIBSC Code No. 90/636 and it is equal to 5.0 IU/ml.

(ii) In-house Evaluation:

Sensitivity and Specificity studies were carried out on samples fresh, as well as frozen, from low risk as well as high risk group. Performance of the test with reference to sensitivity and specificity was evaluated in-house by using a panel containing 2036 negative samples and 210 HIV positive (Ag & Ab) samples.

The results of all the positive and negative samples were compared with commercially available licensed test kit.

The results of the in-house study done are as follows:

No. of Samples	Status	Microlisa HIV Ag & Ab		Commercially Licensed Test	
		Positive	Negative	Positive	Negative
210	HIV Positive	210	-	210	-
2036	HIV Negative	1	2035	-	2036

Sensitivity: 100% **Specificity:** 99.95%

(iii) *External Evaluation:

a) The Kit has been evaluated by NARI (National Aids Research Institute), Pune and the results are:
Sensitivity: 100% **Specificity:** 100%

b) The Kit has been evaluated by NIMHAS (National Institute of Mental and Neuro Sciences), Bangalore and the results are:
Sensitivity: 100% **Specificity:** 100%

**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.*

Precision: Within-run and between-run precisions have been determined by testing 10 replicates of nine samples: two HIV p24 antigen and four HIV antibody positive(four weak, one medium and one strong) and three negative samples. The C.V.(%) of all the samples were within 10%.

20. 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.

21. REFERENCES

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22. 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.	Follow the procedure meticulously & repeat assay.
	b) Inactivated conjugate, improper storage	Check storage of enzyme conjugate and it should be free of any contamination.
	c) Microplate inactivated, due to improper storage	Keep unused strips in aluminium poly pouch with the dessicant pouch inside and proerly closed with clamp & rod.
	d) Inactivated substrate, improper storage or preparation	Use freshly prepared substrate solution. Recheck procedure, repeat assay
	e) Omission of any step in test procedure	Follow the procedure meticulously & repeat assay.
	f) Incorrect (low) incubator temperature, timing or pipetting	Check incubator temperature, procedure & repeat assay
	g) Improper preparation of enzyme conjugate (dilution error) improper mixing of reagents.	Check procedure & repeat assay
2. High O.D. value of Negative control	a) Plate not stopped after 30 minutes of additing stop solution	Follow the procedure meticulously & repeat assay.
	b) Same microtip used for Positive and negative controls	Change micropipette tips while addition of negative/ positive control
	c) Nonspecific attachment/ binding of other reagent	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	Check substrate (TMB Diluent) it should be colourless. If blue in colour then discard and use clean disposable container.
	b) Contaminated washing solution (1X). Poor quality of water used for diluting wash buffer conc.	Check the container and quality of water used for dilution. Use of distilled water is preferred.
	c) Over incubation of substrate and delay in addition of stop solution.	Follow the procedure meticulously.
	d) Insufficient washing. i) Washing not consistent	Check wash device, fill the well close to the top.
	ii) Filling volume not sufficient.	After washing, blot the microwells on absorbent
	iii) Insufficient no. of wash cycles.	tissue. Follow wash protocol meticulously
	iv) Contaminated wash device	
	e) Use of wash solution from other manufacturer.	Use only Microlisa HIV Ag & Ab wash solution.
	f) Working substrate not protected from light	Incubate the plate in dark after addition of substrate.
4. Poor reproducibility	a) Washing problems.	Check all 8 ports/ manifold for uniform flow of wash buffer. If there are blockage, clen the ports.
	b) Uncalibrated pipettes or tips not well fitted, improper pipetting/ dispensing.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	c) Interference in optical pathway due to Air bubbles.	Clean or dry the bottom of microwells, check for bubbles and repeat the readings.
5. False Positive	Beside 3a, b, c, d, e & f incorrect interpretation and calculation of final results	Check the calculation part given in the insert and correctly interpret.
6. False Negative/ low O.D. of Positive control & positive sample	a) Inadequate addition of substrate/conjugate solution.	Follow the procedure meticulously & repeat assay.
	b) Kit expired, reagent of different kit used.	Check the expiry of the kit before use.
	c) White particles in working substrate solution.	Discard the substrate and prepare the working substrate again in fresh tube.
	d) Uncalibrated pipettes, improper pipetting.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	e) Deterioration of Enzyme conjugate	Check storage of Enzyme conjugate. It shall be stored at 2-8°C.
	f) Stop solution is added before 30 minutes. Reaction terminated before 30 minutes.	Follow the test procedure meticulously.
	g) O.D. taken at incorrect wavelength.	Read O.D. values at 450 nm and 630 nm.
	h) Incorrect (low) incubator temperature, timing or pipetting	Check incubator temperature, procedure & repeat assay

in vitro diagnostic Reagent, not for medicinal use

VER-02 R-03

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