HIV Microlisa 3.0

Microwell ELISA Test for the Detection of IgM, IgG & IgA Antibodies to HIV-1 (Including Subtype C & Group O) 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 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.

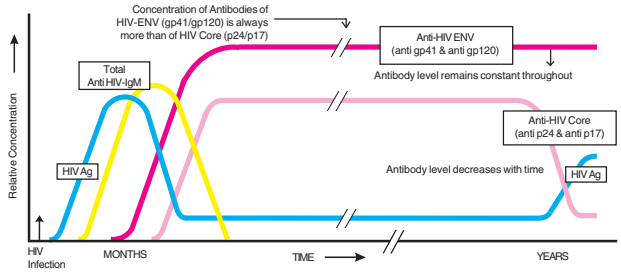


Fig.1

HIV MICROLISA 3.0 is developed to detect anti-HIV ENV (envelope) antibodies to HIV-1 and / or HIV-2 with equal reactivity.

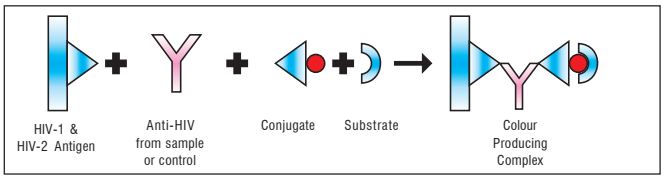
It has been observed that the core protein of HIV-1 and HIV-2 show cross reactivity whereas envelope proteins are more type specific and moreover antibodies against the envelope gene products can be found in almost all infected people. HIV Microlisa 3.0 has been developed and designed to be extremely 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.

2. INTENDED USE

HIV Microlisa 3.0 is an in-vitro qualitative enzyme immunoassay for the detection of IgM, IgG & IgA antibodies to HIV-1 and / or HIV-2 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

HIV Microlisa 3.0 test is an enzyme immunoassay based on Indirect ELISA.



HIV envelope proteins proteins gp41, C terminus of gp 120 for HIV-1, and gp 36 for HIV-2 representing immunodominant epitopes are coated onto microtiter wells. Specimens and controls are added to the microtiter wells and incubated. Antibodies to HIV-1 and HIV-2 if present in the specimen, will bind to the specific antigens absorbed onto the surface of the wells. The plate is then washed to remove unbound material. Horseradish peroxidase (HRP) conjugated gp41, C ter of 120 of HIV-1 and gp36 of HIV-2 is added to each well. This conjugate will bind to HIV antigen-antibody 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 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 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 British and European Standard BS EN 980:2003.

	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

5. PACK SIZE

- 96 Tests

6. COMPONENTS IN EACH HIV MICROLISA 3.0 KIT

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

HIV Microlisa 3.0 Strip Plates	12 Strips (12 X 8 wells) Breakway microwells coated with HIV-1 & HIV-2 recombinant proteins 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.) gp41, C ter of 120 of HIV-1 and gp36 of HIV-2 conjugated with horseradish peroxidase with protein stabilizers.
Conjugate Diluent	1 Bottle (15 ml.) Buffer containing protein 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 with TMB diluent before use.
TMB Diluent	1 Bottle (10 ml.) Buffer solution containing H_2O_2 with preservative
Control —	1 Vial (2.0 ml.) Ready to use, normal human serum negative for HIV, HCV, and HBsAg.
Control +	1 Vial (2.0 ml.) Ready to use, inactivated and diluted human serum; positive for HIV antibodies and non-reactive for HBsAg and HCV, contains sodium azide as preservative.
Stop Solution	1 Bottle (15 ml.) Ready to use, 1N sulfuric acid.
Plate Sealers	Adhesive backed sheets for sealing microwell plate/strips.

7. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

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

8. SPECIMEN COLLECTION & PREPARATION

1. Only human serum or plasma samples should be used for the test. While preparing serum samples, remove the serum form 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. Use of heat inactivated, icteric hyperlipemic and hemolyzed and Icteric hyperlipemic samples should be avoided as may give erroneous results.

9. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

HIV Microlisa 3.0 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.

10. 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. Controls and Sample diluent 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. 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 $\pm$ 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 as Bottle of TMB Substrate & Diluent are of same size.
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. Use separate tips for TMB Substrate and TMB diluent.
19. Avoid strong light exposure during the assay.
20. 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.
21. 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.
22. 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 at 37°C.

1. HIV Microlisa 3.0 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 & two positive controls should be included in the run while opening the fresh kit. However for one or two strips, two negative and one positive control and for more strips at least three 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.

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. 25 X wash buffer concentrate with 480 ml. of distilled or deionized water. 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 4 additional times for a total of 5 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.

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 HIV Microlisa 3.0 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 50 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 Positive Control in D-1 & E-1 wells.
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 30 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 after blanking A-1 well. (Bichromatic absorbance measurement with a reference wavelength 600 - 650 nm is recommended when available).

15. SUMMARY OF PROCEDURE*

Add Sample Diluent		Sample Diluent 50 μl
Add control & samples		100 μl
Cover the plate & incubate		30 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 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 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.

* RTU - Ready to use

16. CALCULATION OF RESULTS

Abbreviations

NC - Absorbance of the Negative Control

NC$\bar{x}$ - Mean Negative Control

PC - Absorbance of the Positive Control

PC$\bar{x}$ - Mean Positive Control

TEST VALIDITY:

Negative Control Acceptance Criteria:

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

Positive Control Acceptance Criteria:

1. PC must be $\geq$ 0.50

CUT OFF VALUE

Absorbance

NC	-	0.042 A1 Well	PC	-	1.412	D1 Well
	-	0.040 B1 Well		-	1.392	E1 Well
	-	0.041 C1 Well	Total :		2.804	2 Wells
Total :		0.123 3 Wells				

NC$\bar{x}$ = 0.123/3 = 0.041 PC$\bar{x}$ = 2.804/2 = 1.402

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

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

Where NCx 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.
2. Test specimens with absorbance value greater than or equal to the cut off value are reactive for anti-HIV by HIV Microlisa 3.0.

Note: Test specimens with absorbance value within 10% below the cut off should be considered suspect for the presence of antibodies, 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 HIV Microlisa 3.0. 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 HIV Microlisa 3.0. 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. HIV Microlisa 3.0 assay is designed for testing antibodies against HIV-1 and/or HIV-2 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, HIV Microlisa 3.0 testing alone cannot be used to diagnose AIDS even if antibodies 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. 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, Tuberculosis, Mycobacterium avium, Herpes simplex, Hypergammaglobulinemia, Malignant neoplasms, Rheumatiod arthritis, Tetanus vaccination, Autoimmune diseases, Blood Transfusion, Multiple myeloma, Haemophelia, Heat treated specimens, Lipemic serum, Anti-nuclear antibodies, T-cell eukocyte antigen antibodies, Epstein Barr virus, HLA antibodies and other retroviruses.

19. PERFORMANCE CHARACTERISTICS

The performance of HIV MICROLISA 3.0 has been evaluated in house with fresh as well as frozen 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 HIV. The results of in-hoouse studies are as follows:

No. of Samples	Status	HIV Microlisa 3.0 + ve	HIV Microlisa 3.0 - ve
167	All ELISA +ve	167	-
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 three specimens : a negative, a low positive and a strong positive. The C.V.(%) of negative, low positive and strong positive values 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.

NOTICE: Every effort is made to supply ordered consignment as per the sample submitted but due to continuous development, the company reserves the right to improve/change any specifications/ components without prior information/notice to the buyer.

21. REFERENCES

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5. Hofbauer M.J. et al Journal of Clinical Microbiology, (1988) 26(1); 116 "Comparison of Western Blot (Immunoblot) based on Recombinant-Derived p41 with Conventional Tests for serodiagnosis of Human Immunodeficiency Virus infections"
6. Mayer Kwnnwrh H. et al. The American Journal of Medicine, (1987) 83;208 "Correlation of Enzyme-Linked Immunosorbant Assays for Serum Human Immunodeficiency Virus Antigen and Antibodies to Recombinant Viral.

22. 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	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
2.	No colour or light colour developed at	a) Any one reagent has been added in wrong sequence.	Check procedure and repeat assay.
	the end of 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 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 HIV Microlisa 3.0 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.	1e.
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) Inadequate addition of substrate/conjugate solution	Recheck the test procedure and reagent volume.
		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.

IVD

REV.: 00

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