3. Dawson G.J., et al. The Journal of the Infectious Diseases, (1988) 157 (1); 149 "Reliable Detection of Individuals Seropositive for the Human Immunodeficiency Virus (HIV) by competitive Immunoassays using Escherichia coli-Expressed HIV Structural Proteins."

4. Gallo R.C., Science (1984) 224;500 "Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and at Risk for AIDS."

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".

19. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Controls out of validation limit	a) Controls/ Calibrator deterioration due to improper storage or used after expiry.	Ensure calibration is done after 15 days and use controls/ Calibrator within 30 days once opened and check storage temp. It should be 2-8ºC.
	b) Cross contamination of Controls	Pipette carefully and do not interchange caps.
	c) Reagents deterioration to improper storage or used after expiry.	Use reagents within 30 days once opened and Check storage temp. It should be 2-8ºC.
	d) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.
2. False Positive test results	a) Use of turbid, lipaemic or hemolyzed sample.	Use clear fresh sample. Refer test specimen collection, handling and processing for more details.
	b) Sample position is wrongly defined while loading the sample details in analyzer.	check the sample position and run the test meticulously.
	c) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.
	d) Wrong Sample identification	Make sample I.D. at the time of sample Collection.
3. False negative test results	a) Sample deterioration due to improper Storage or microbially contaminated sample.	Use clear fresh sample immediately after collection. Refer Specimen collection, and handling processing for more details.
	b) Sample position is wrongly defined while loading the sample details in analyzer.	check the sample position and run the test meticulously.
	c) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.
	d) Wrong sample identification.	Mark the sample I.D. at the time of sample collection.

in vitro diagnostic Reagent, not for medicinal use

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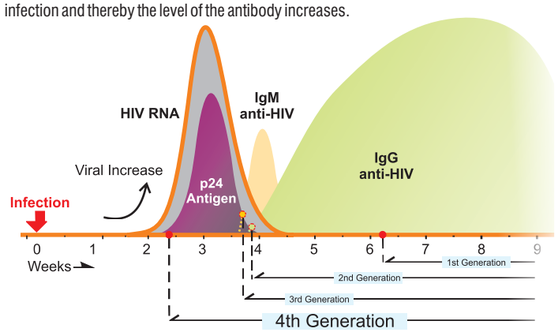
HIV Ag & Ab *i*Clia

Chemiluminescence microparticle immunoassay for Detection of HIV-1 p24 antigen and Antibodies to HIV-1 (Including Subgroup O & C) and HIV-2 in Human Serum/ Plasma

1. INTRODUCTION

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.



HIV Ag & Ab iClia 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. HIV Ag & Ab iClia 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

HIV Ag & Ab iClia is a chemiluminescence microparticle immunoassay designed for in vitro qualitative detection of antibodies to HIV-1 and / or HIV-2 and HIV -1 P24 antigen in human serum or plasma and is used as a screening test for testing of collected blood prior to transfusion. This kit is only operational in conjuction with J. Mitra CLIA Analyzer.

3. PRINCIPLE

HIV Ag & Ab iClia is chemiluminescence microparticle immunoassay based on the "Direct Sandwich" principle. The magnetic microparticles are coated with anti-HIV p24 antibody and HIV-1/-2 specific antigen. The samples are added in the wells followed by addition of AE conjugate (anti-HIV p24 antibody and HIV-1/-2 specific antigen linked to acridinium ester). A sandwich complex is formed wherein HIV-1/2 antibody and / or HIV p24 antigen (from serum sample) is "trapped" or "sandwiched" between the microparticles coated anti-p24 antibody and HIV-1/2 antigen and anti-p24 & HIV-1/2 antigen labelled with AE conjugate. Unbound conjugate is then washed off with wash buffer. The amount of bound AE conjugate is proportional to the concentration of HIV-1/2 antibody and / or HIV p24 antigen present in the sample. Finally pre-trigger and trigger solution containing hydrogen peroxide and sodium hydroxide solution is added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative Light units (RLUs). There is a direct relationship between the amount of HIV-1/2 antibody and / or HIV p24 antigen present in the sample and the RLUs detected by the optical system. Results are calculated automatically based on the cut-off value.

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 European Standard EN ISO 15223-1:2021.

	Manufactured By	IVD	In vitro diagnostic medical device
Σ	No. of tests		Instruction for use
LOT	Lot Number Batch Number	2ºC 8ºC	Temperature Limitation
	Manufacturing Date		Caution, see instruction for use
	Expiry Date	REF	Catalogue Number
	Keep away from sunlight		Do not use if package is damaged
BIO	Contains biological Material of Human Origin	BIO	Contains biological Material of Animal Origin
IN	Country of Manufacture		Keep Dry

5. KIT PRESENTATION

- 50 Tests
- 100 Tests
- 150 Tests

6. KIT & ITS COMPONENTS

COMPONENT	DESCRIPTION
Microparticle Buffer (RA)	Magnetic microparticles coated with anti-HIV p24 antibody and HIV-1/-2 specific antigen with preservatives.
Assay Buffer (RB)	Assay Buffer containing BSA with preservatives.
Diluent (RC)	Buffer containing protein stabilizers & antimicrobial agents as preservative.
AE Conjugate (RD)	Containing Anti-HIV p24 antibody and HIV-1/-2 specific antigen linked to acridinium ester with preservatives.
Calibrator-1 (C0)	Cut-off calibrator, BSA in buffer with preservatives.
Calibrator-2 (C1)	Cut-off calibrator, HIV antibodies in buffer with preservative.
Control-1 (Q1)	Normal human plasma negative for HIV, HCV and HBsAg with preservatives.
Control-2 (Q2)	Positive for HIV antibodies and non-reactive for HBsAg and HCV antibodies with preservatives.
Reagent Plugs	Silicon caps to cover the opened reagents.

7. STORAGE AND STABILITY

The kit should be stored at 2-8ºC in the cool and driest area available. Expiry date on the kit indicates the date beyond which kit and its components should not be used. **Once the kit is opened, onboard stability of reagents, calibrator and control is 30 days at 2-8ºC.**

8. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

- **Pre-Trigger Solution:** Hydrogen peroxide solution.
- **Trigger Solution:** Sodium hydroxide solution.
- **Wash Buffer:** Phosphate buffered saline solution with surfactant.
- **Assay Cup**
- **J. Mitra CLIA Analyzer**

All materials and analyzer to be used for running the HIV Ag & Ab iClia shall be from J. Mitra & Co. Pvt. Ltd.

9. SPECIMEN COLLECTION & HANDLING

1. Only human serum or plasma samples should be used for the test.
2. For serum collection use serum vacutainer. While preparing serum samples, remove the serum from the clot as soon as possible to avoid hemolysis. Fresh serum/plasma samples are preferred.
3. For plasma collection: use Dipotassium EDTA, Tripotassium EDTA, Sodium heparin and lithium heparin gel vacutainer and CPDA blood bag.
4. Specimens should be free of microbial contamination and may be stored at 2-8ºC for one week, or frozen at -20ºC or lower. Avoid repeated freezing and thawing.
5. Do not use heat inactivated samples as their use may give false results. Hemolyzed and Icteric hyperlipemic samples may give erroneous results.
6. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
7. Always use clear specimens. Centrifuge viscus/ thick or turbid specimen at 10,000 RPM for 15 minutes or 4,000 RPM for 30 minutes prior to use to avoid inconsistent result.
8. Use of disposable pipettes or pipette tips is recommended to prevent cross contamination.

10. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

HIV Ag & Ab iClia 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. Centrifuge the sample at 10,000 rpm for 15 minutes or 4,000 rpm for 30 minutes.

(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.

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. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
7. 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 121ºC at 15psi for 60 minutes. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
8. 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.
9. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.

12. PRECAUTIONS FOR USE & REAGENT HANDLING

1. Do not use kit components beyond the expiration date which is printed on the kit.
2. Store the reagents & samples at 2-8ºC.
3. Do not pool reagents from within a batch or between different batches, as they are optimised for individual batch to give best results.
4. Before loading the reagent kit in the clia analyzer for the first time, ensure proper mixing of microparticle bottle to resuspend microparticles that may have settled during transport or storage.
5 Once reagents are opened, reagent Plug must be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if reagent plugs are not used according to the instructions given.
6 To avoid contamination, wear clean gloves when placing a reagent plug on an uncapped reagent bottle.
7. Once a reagent plug has been placed on an open reagent bottle, do not invert the bottle as this will result in reagent leakage and may compromise assay results.
8. Reagents may be stored on or off the Chemiluminescence immunoassay analyzer. If reagents are removed from the analyzer, store them at 2-8°C (with Reagent Plugs) in an upright position. For reagents stored off the system, it is recommended that they should be stored in their original trays and boxes to ensure they remain upright. If the microparticle bottle does not remain upright (with a Reagent Plug placed) while in refrigerated storage off the system, the reagent kit must be discarded.
9. Run control-1 & control-2 in each assay to evaluate validity of the kit.
10. Distilled or deionised water must be used for wash buffer preparation.
11. Avoid strong light exposure during the assay.
12. In case of any doubt the run should be repeated.

13. TEST PROCEDURE

Assay Procedure

1. Refer to the Clia Analyzer user manual for detailed information on preparing the analyzer.
2. Before loading the HIV Ag & Ab iClia reagent kit on the analyzer for the first time, mix contents of the microparticle bottle to resuspend microparticles that may have settled during transporation/ storage. Once the microparticles have been loaded, no further mixing is required.

Important Note: Swirl the microparticle (RA) bottle 30 times. Visually inspect the bottle to ensure microspheres are resuspended. If microspheres are still adhered to the bottle, continue to Swirl the bottle until the microspheres have been completely resuspended. If the microspheres do not resuspend, DO NOT USE. Once the microspheres have been resuspended, remove the cap and place the the reagent plug on the bottle to make it ready to use. Remove the cap of (RA), (RB), (RC) and (RD) bottles and place the reagent plugs before use as follow:

(RA) & (RB) : Natural color plug
(RC) : Purple color plug
(RD) : Brown color plug

3. Load the HIV Ag & Ab iClia reagent tray on the Chemiluminescence immunoassay analyzer.
4. Verify that all necessary reagents are available in the reagent tray.
5. Ensure that adequate sample volume (not less than 250 μL) is present in sample tube prior to running the test.
6. Sample volume required for each additional test from same sample tube is 50 μL.
7. Ensure sample positions are properly defined at the time of loading in the analyzer.
8. The HIV Ag & Ab test-specific parameters are stored in reagent barcode placed on the reagent tray and read through barcode reader. In case, the barcode cannot be read, contact customer support at: 011-47130300, 500 or write us at: jmitra@jmitra.co.in.
9. Run calibration as mentioned in heading calibration below.
10. **Mix HIV Ag & Ab iClia calibrator and controls by gentle inversion before use.** Open the the cap and place the calibrator and control-1 & control-2 vials into each respective sample positions. Read the barcode for calibrator and controls provided with the kit.
11. Run calibration as mentioned in heading calibration below.
12. Press Run. The test result for first sample will be obtained at 45 minutes.
13. The Chemiluminescence immunoassay analyzer performs all the functions automatically and calculate the results.

Calibration

1. Test Calibrator in triplicate. Both control-1 and control-2 must be tested in each run to evaluate the assay calibration. Ensure that calibrator and controls values are within the validity range specified in this instruction manual.
2. Once calibration is accepted (within range) and stored, all subsequent samples may be tested without further calibration unless, recalibration is required.
3. Recalibrate the analyzer in following conditions:
   a) After each exchange/use of new lot (Test reagent and pre-trigger/ Trigger solution/wash buffer).
   b) Every 15 days and/or at the time of any component to be changed.
   c) Controls are out of validation range.
   d) Required by pertinent regulations.
   e) After specified service procedures have been performed or maintenance to critical part or subsystems that might influence the performance of the HIV Ag & Ab iClia.

TEST VALIDITY:

Ensure the following is within specified acceptance criteria

i) Sample to cut-off ratio (S/CO) of control-1 (Q1) must be $\leq$ 0.5. If it is not so, the run is invalid and must be repeated or calibrated. **Exact target value with SD value is lot specific and mentioned in QC bar code.**

ii) Sample to cut-off ratio (S/CO) of control-2 (Q2) must be > 2.0. If it is not so, the run is invalid and must be repeated or calibrated. **Exact target value with SD value is lot specific and mentioned in QC bar code.**

iii) Sample to cut-off ratio (S/CO) of calibrator -1 (C0) must be between 0 - 0.5. If it is not so, the run is invalid and must be repeated or calibrated.

iv) Sample to cut-off ratio (S/CO) of calibrator-2 (C1)must be between > 0.8. If it is not so, the run is invalid and must be repeated or calibrated.

Note: If one of the Calibrator-1 (C0) &/or Calibrator-2 (C1) individual values differ from other 2 replicates ,then analyser automatically disregard that value and calculate the calibrators value with the two remaining calibrator values and provide the result.

RESULT CALCULATION:

The analyzer automatically calculates the sample to cut-off ratio (S/CO) of each sample based on cut-off value using formulas.

a. Cut off value = mean RLU of calibrator-1 + mean RLU of calibrator-2 x Factor (F)

b. Calculation of Sample to cut-off Ratio : Sample cut-off Ratio is calculated as follows:

Sample cut-off Ratio (S/CO) = RLU of Sample/ Cut-off value

Note: Factor (F) is batch specific and is provided in the calibrator barcode.

14. INTERPRETATION OF RESULTS

a. If the HIV Ag &/or Ab S/CO is < 1.0 then interpret the sample as Negative for HIV Ag &/or Ab.

b. If the HIV Ag &/or Ab S/CO is > 1.0 then interpret the sample as Positive for HIV Ag &/or Ab.

15. 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 25.0 IU/ml.

ii) Diagnostic Sensitivity and Specificity: The Performance of the HIV Ag & Ab iClia with reference to sensitivity and specificity was evaluated in-house with the panel of 78 negative and 15 HIV Ag & Ab positive samples. The performance is also checked with fresh clinical negative (236) and HIV Ag & Ab Positive (20) 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	HIV Ag & Ab iClia		Commercially available HIV Ag & Ab ELISA	
		Positive	Negative	Positive	Negative
35	HIV Positive	35	0	35	0
314	HIV Negative	0	314	0	314

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

C) Analytical Specificity :

The analytical specificity of the HIV Ag & Ab iClia Test kit is checked to check the potential for false results with 12 cross-reacting specimen; HIV, HCV, RA, ASO, CRP and antenatal. The specificity on all above samples tested is 100%. The analytical specificity of the test kit is also checked with potentially interfering substances /samples to check the potential for false results arising from interference from potentially interfering substance .There was no interference with the test results when biomolecules; Bilirubin (20mg/dl), Hemoglobin (500mg/dl), Triglyceride (1000mg/dl), Total protein(10mg/dl), RF(1000mg/ml), ANA(400mg/ml) & HAMA positive human plasma(600mg/mL) were added to the test specimen with much higher level in normal human blood.

Precision: PrPrecision is checked by running HIV Ag & Ab iClia test in 10 replicates (Intra assay variation, Inter assay variation) and Inter Machine variation with Kit controls(Contro 1& Control 2) , 2 HIV positive samples; one strong positive and one weak positive .The CV% in Sample RLU to Cutoff ratio (S/CO) of both the controls and positive samples is within 10% for intra-assay variation and within 15% for inter-assay and inter-machine variation.

16. LIMITATION OF THE TEST

1. HIV Ag & Ab iClia assay is designed for testing antibodies against HIV-1 and/or HIV-2 and HIV-1 p24 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.
2. In establishing infection of HIV-1 and/or HIV-2 or, in evaluating patients with AIDS symptoms, HIV Ag & Ab iClia 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.
3. **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.
4. Some samples show cross reactivity for HIV antibodies. **Following factors are found to cause false positive HIV antibody test results:** Presence of Rf antibodies, patients with auto-immune disease, liver problems, renal disorders, antenatal samples, Naturally occurring antibodies, Passive immunization, Leprosy, Tuberculosis, Mycobacterium avium, Herpes simplex etc.

17. 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.

18. REFERENCES

1. Busch M. P. et al. Transfusion 31 (2): 129 "Reliable Confirmation and Quantitation of Human Immunodeficiency Virus Type 1 Antibody using a Recombinant Antigen Immunoblot Assay."
2. Chang N. T., Science (1982) 228;92 "Expression in Escherichia coliof Open Reading Frame Gene Segments of HTLV-III