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Immunoassay

REF CMJ0102

100 tests

Anti-HIV CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the qualitative determination of antibody to HIV (human immunodeficiency virus) in human serum and plasma (EDTA, heparin or sodium citrate).

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Key to Graphical Symbols Used

Symbol	Meaning	Symbol	Meaning
LOT	batch code		use by
	Manufacturer		contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use

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IVD

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Contact your local dealer for all product-related questions in your local language

Introduction

Two types of human immunodeficiency virus, HIV-1 and HIV-2, have been described and implicated as causative of the Acquired Immunodeficiency Syndrome (AIDS) [1, 2]. Both are retroviruses which are transmitted by sexual contact, exposure to blood or blood products, and prenatal or perinatal infection of a fetus or newborn[3]. Antibodies against HIV are neatly always detected in AIDS patients and HIV-infected asymptomatic individuals[3, 4]. HIV has several major genes coding for structural proteins that are found in all retroviruses as well as several nonstructural genes unique to HIV. The HIV genome contains three major genes, 5'gag-pol-env-3', encoding major structural proteins as well as essential enzymes[5]. These are synthesized as polyproteins which produce proteins for virion interior, called Gag, group specific antigen; the viral enzymes (Pol, polymerase) or the glycoproteins of the virion *env* (envelope) [6].
Knowledge on genetic variability of the HIV virus strains were acquired by sequencing the GAG, POL, and ENV genes of the representative strains of each subtype. The HIV-2 virus includes 5 sub-types. Some HIV-1 variants have only 70% homology for the GAG and POL genes with the main isolates and only 50% for the ENV gene, these differences can explain the failure of the infection diagnosis in some patients[7]. HIV/AIDS pandemic is a complex mix of diverse epidemics within and between countries and regions of the world, and is undoubtedly the defining public-health crisis of our time. As of 2012, approximately 35.3 million people are living with HIV globally[8]. Of these, approximately 17.2 million are men, 16.8 million are women and 3.4 million are less than 15 years old[8]. There were about 1.8 million deaths from AIDS in 2010, down from 2.2 million in 2005[8].
The Anti-HIV CLIA Microparticles assay is a chemiluminescent microparticle immunoassay (CMIA) for the simultaneous qualitative detection of human immunodeficiency virus (HIV) antibodies to HIV type 1 (HIV-1 Group M) and/or type 2 (HIV-2) in human serum and plasma (EDTA, heparin or sodium citrate). This assay is intended to be used as an aid in the diagnosis of HIV-1/HIV-2 infection.

Measurement Principle

This assay is based upon the two-step sandwich method. The sample, HIV-1/HIV-2 antigen coated microparticles and Sample Diluent are combined. HIV-1/HIV-2 antibodies present in the sample binds to HIV-1/HIV-2 antigen coated microparticles. After washing, Enzyme Conjugate is added. During the incubation, a complex is generated among the solid phase, the Anti-HIV within the sample and enzyme-labeled antigen by immunological reactions. Chemiluminescent Substrate is added and the complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of Anti-HIV in the samples.

Materials provided

1. Positive Control

1 vial containing 1.0 ml of Tris-HCl buffer, containing heat-inactivated human plasma positive for HIV-1/HIV-2 antibody and proteins of bovine origin and 0.1% ProClin 300® preservative. The plasma is nonreactive for HCV, HBsAg and Syphilis.
Reagent provided ready to use.

2. Negative Control

1 vial containing 1.0 ml of PBS buffer, containing proteins of bovine origin. Contains 0.1% ProClin 300® preservative. The negative control is nonreactive for HBsAg, HIV-1 and HIV-2, HCV and Syphilis.
Reagent provided ready to use.

3. Reagent pack

Reagent pack provided ready to use.

- Microparticles Solution

1 vial containing 2.3 ml of HIV-1 antigen (HIV-1 gp41) and HIV-2 antigen (HIV-2 gp36) coated microparticles in Tris-HCl buffer containing BSA. Contains a selection of preservatives.

- Enzyme Conjugate

1 vial containing 11.0 ml of horseradish-peroxidase labeled HIV-1/HIV-2 antigen in Tris-HCl buffer containing proteins of bovine origin. Contains ProClin 300® preservative.

- Sample Diluent

1 vial containing 5.5 ml of Tris buffer containing casein. Contains a selection of preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000
- AutoLumo A2000 Plus

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 or AutoLumo A2000 Plus.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample tube(s) or cup(s) for sample containing
4. Chemiluminescent Substrate
5. System Wash for washing the pipetting needle
6. Wash Buffer used in washing procedure
7. Distilled or deionized water

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: the positive control contains material of human origin, which has been tested and found non-reactive for HCV, Syphilis and HBsAg, but reactive for HIV-1/HIV-2. It is recommended that all materials of human origin be considered potentially infectious. This assay contains materials of animal origin. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported.
6. Some reagents contain ProClin 300® may cause sensitization by skin contact, which must be avoided to contact with skin. This material and its container must be disposed in a safe way. If swallowed, seek medical advice immediately and show this container or label.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
9. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
10. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
11. Do not use reagents beyond the labeled expiry date.
12. Do not mix or use components from kits with different batch codes.
13. When storing the controls, be certain the vials are securely sealed.
14. Ensure the microparticles are resuspended before loading it on the analyzer.

15. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
16. Do not substitute any reagent in this kit from other manufacturers or other lots.
17. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store the kit at 2-8℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10℃ for a minimum of 2 hours prior to use.
3. Store the reagents pack upright at 2-10℃ on the analyzer. They may be stored on the analyzer for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-8℃ in an upright position. For reagents stored off the analyzer, it is recommended that they be stored in their original trays and boxes to ensure they remain upright.
4. Once the reagent pack is open, it can be stored at 2-8℃ for 1 month.
5. Seal and return the remaining Positive or Negative Control to 2-8℃, under that conditions the stability will be retained for 1 month, for longer use, store opened controls in aliquots and freeze at -20℃. Avoid multiple freeze-thaw cycles.

Sample

1. Collect samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Do not use samples with obvious microbial contamination.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy etc. in appearance prior to use to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
12. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials

- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit

- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests

- Place the sample tube(s) or cup(s) on the sample holder, 100 μl of sample for each test. But consider the sample container and 150 μl of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample holder and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticles Solution and Sample Diluent to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine Anti-HIV in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibration

- Analyzer can read the bar code on the reagent pack automatically to obtain the essential information for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the Anti-HIV CLIA Microparticles Positive and Negative Controls into the sample tube (s) or cup(s) and place them on the sample holder. They are automatically tested in triplicate or duplicate (triplicate for Positive Controls and duplicate for negative controls) at the beginning of each batch. The Assay Analyzer system will not generate results when controls values do not meet specifications. This may indicate either deterioration or contamination of reagents, or analyzer failure.
- Load the sample holder and input Negative & Positive Control information on the system software interface.
- Select "run" to start the test, calibration is required every 28 days.
- Once the control results is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and Chemiluminescent Substrate with new batch

code is used
    - Beyond the expiration date of calibration
    - Important parts of the analyzer are replaced or repaired
- Refer to the Assay Analyzer's operation manual.

Measurement Results

- Calculation of Cut-off and S/CO Values

The Assay Analyzer system calculates the Anti-HIV CLIA Microparticles assay Cut-off value using the following formula:
Cut-off Values = PCx * 0.4
PCx: the mean of the positive control RLU replicates
Example: Mean PC RLU = 5922014
Cut-off Value = 5922014 x 0.4 = 2368805

The Assay Analyzer system calculates the Anti-HIV CLIA Microparticles assay S/CO for each sample using the following formula:
S/CO = Sample RLU/Cut-off Value
Example: Sample RLU =3326910
S/CO = 3326910/2368805 = 1.40

- Interpretation of Results

    - In the Anti-HIV CLIA Microparticles assay, samples with S/CO values of less than 1.00 are considered nonreactive and need not be tested further. Nonreactive samples are considered negative for Anti-HIV by the criteria of Anti-HIV CLIA Microparticles.

    - Samples with an S/CO value of greater than or equal to 1.00 are considered initially reactive by the criteria of the Anti-HIV CLIA Microparticles assay. All samples that are reactive on initial testing must be centrifuged prior to retesting. Initially reactive samples must be retested in duplicate using the Anti-HIV CLIA Microparticles Assay Kit.

    - If the sample RLU for both retests is less than the Cut-off value, the sample is nonreactive. Nonreactive samples are considered negative for Anti-HIV by the criteria of the Anti-HIV CLIA Microparticles.

    - If the sample RLU for either duplicate retest is greater than or equal to the Cut-off value, the sample is considered repeatedly reactive. Repeatedly reactive results indicate the presence of Anti-HIV by the criteria of the Anti-HIV CLIA Microparticles.

    - Although the association of infectivity of donated blood or plasma and the presence of Anti-HIV is strong, it is recognized that presently available methods for Anti-HIV detection are not sensitive enough to detect all potentially infectious units of blood, plasma, or possible cases of HIV infection. A nonreactive test result does not exclude infection.

Control Procedure

The recommended control requirement for this assay involves using Positive and Negative Controls to verify assay performance. The result is valid if the control results meet assigned specifications (Mean PC RLU/ Mean NC RLU＞10). When a control fails to meet assigned specifications, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. It is recommended that each laboratory establish its accepted range to ensure proper test performance.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. False-reactive test results can be expected with any test kit. False-reactive test results have been observed due to nonspecific interactions.
4. Some samples that have undergone multiple freeze-thaw cycles or have been stored frozen for prolonged periods may result in erroneous or inconsistent test results.
5. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
6. Performance of this test has not been established with neonatal samples.
7. Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human Anti-mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
8. This assay was designed and validated for use with human serum and plasma from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
9. Due to limitation of methodology or immunological specificity and other reasons, the test results from different manufacturers' reagents for the same sample may be different, so such results should not be directly compared with each other, so as to avoid the wrong medical explanation. It is recommended that the characteristics of the different manufacturers' reagents should be indicated when reported to the clinician.

Performance Characteristics

1. Measurement Precision

Assay reproducibility was determined during the clinical evaluation of the Anti-HIV CLIA Microparticles using three lots of reagents. The three human serum based panel members (Low, Medium and high value members) were tested using 3 batches of reagents, in replicates of 10 once daily for three days. This assay is designed to have a within-run precision and a between-run precision with a <15% coefficient of variation (CV). The Coefficients of Variation (CVs) were calculated based upon the variance components. Data from this study are summarized in the following table.

Panel Member	No.	Within-run Precision			Between-run Precision		
		Mean	SD	%CV	Mean	SD	%CV
1	30	9.05	0.38	4.2%	8.73	0.44	5.0%
2	30	4.87	0.11	2.2%	4.75	0.24	5.0%
3	30	2.17	0.10	4.7%	2.17	0.11	5.2%

2. Clinical Sensitivity

A total of 1142 serum and plasma samples from individuals known to be positive for HIV antibodies were tested with the Anti-HIV CLIA Microparticles assay. Of the 1142 samples, all samples were repeatedly reactive, clinical sensitivity of this assay is 100.00%.

3. Clinical Specificity

A total of 6182 fresh serum and plasma samples from volunteer blood donors and medical examination donors were collected and tested at different geographically distinct blood centers and hospitals. These sample donors include 150 pregnant individuals, 50 children and 5902 normal individuals. Of the 6182 samples, 11 (6171/6182) samples were repeatedly reactive, and based on supplemental test results from a licensed and/ or research immunoblot assay, they were Anti-HIV negative. Clinical specificity of this assay is 99.82%.

4. Analytical Specificity

Cross reaction: this assay was evaluated for potential cross-reactivity for samples from individuals with medical conditions unrelated to HIV infection. The samples were tested using the Anti-HIV CLIA Microparticles assay. The data are summarized in the following table.

Category	No.	Anti-HIV CLIA Microparticles assay	
		Reactive	Nonreactive
Anti-EBV positive	9	0	9
Anti-HBV positive	9	0	9
Anti-HCV positive	9	0	9
Rh.[a] factor positive	9	0	9
Rubella	9	0	9
Syphilis	9	0	9
Anti-CMV positive	9	0	9

a. Rh.[a] factor: Rheumatoid factor

Interference: at the concentrations listed below, bilirubin (conjugated and unconjugated), haemoglobin and triglycerides showed less than 10% interference in the Anti-HIV CLIA Microparticles assay for 10 negative samples and 10 positive samples:

- Bilirubin ≤40 mg/ dL
- Haemoglobin ≤100 mg/dL
- Triglycerides ≤3000 mg/dL

5. Tube Type Matrix Study

The following tube types are acceptable for use with this assay:

* Serum and serum separator
* EDTA, heparin or sodium citrate plasma

Different sample types were taken from 31 individuals. The tube types listed in the table below showed no significant difference between serum and plasma.

With the tube types listed in the table below, Anti-HIV CLIA Microparticles assay showed the S/CO values for different tube type matrix.

Tube type matrix	Serum	Heparin Plasma	EDTA Plasma	Sodium Citrate Plasma
Mean S/CO	0.10	0.08	0.09	0.08
Maximal S/CO	0.05	0.02	0.04	0.02

6. Performance on BBI Anti-HIV 1/2 Seroconversion Panel

BBI PRB 966 Anti-HIV 1/2 Seroconversion Panel

PRB966 Panel member	Days since 1st bleed	BBI Ref. Data Immunoassay Result, S/CO		This Assay
		Assay 1	Assay 2	
PRB966-01	0	0.3	0.4	0.1
PRB966-02	2	0.3	0.4	0.1
PRB966-03	20	0.3	0.4	0.1
PRB966-04	22	0.3	0.4	0.0
PRB966-05	30	0.3	0.4	0.1
PRB966-06	35	0.3	0.4	0.1
PRB966-07	37	0.3	0.5	0.1
PRB966-08	44	0.5	0.7	4.1
PRB966-09	48	5.2	3.3	79.8
PRB966-10	51	26	15.6	202.7

The results showed 4 days earlier detection of Anti-HIV CLIA Microparticles assay comparing 2 other CE marked commercial kits.

Literature References

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