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Immunoassay

REF CMJ0104/CMJ0103/CMJ0105/CMJ0106/CMJ0107

50 tests*1/100 tests*1/100 tests*2/100 tests*5/50 tests*2

HIV Ag/Ab Combo CLIA Microparticles

This HIV Ag/Ab Combo CLIA Microparticles assay is a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the qualitative detection of human immunodeficiency virus (HIV) p24 antigen and antibodies to HIV type 1 (HIV-1 group M and group O) and/or type 2 (HIV-2) in human serum and plasma. This assay is intended to be used as an aid in the diagnosis of HIV-1/HIV-2 infection and as a screening test for donated blood and plasma.
This test reactive result does not distinguish between the detection of HIV p24 antigen, HIV-1 antibody, or HIV-2 antibody.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	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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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 HIV structural protein most often used as the marker of antigenemia is the core protein, p24. p24 antigen tests are used for early detection of HIV, as p24 antigen rises soon after infection relative to antibodies, and the test is often used in combination with an antibody test to effectively cover a longer portion of the window period.

Measurement Principle

This assay is based upon the principle of the two-step sandwich technique for the qualitative detection of the various antibodies to HIV-1 (Group M and Group O) /HIV-2 virus and p24 antigen in human serum or plasma, using chemiluminescent microparticle immunoassay technology. In the first step, sample, microparticles solution and biotin labeled p24 antibody solution are added. HIV-1/HIV-2 antibodies present in the sample bind to the HIV-1/HIV-2 antigen coated microparticles, HIV p24 antigen present in the sample bind to the HIV p24 antibody coated microparticles and biotin labeled p24 antibody. After washing, enzyme conjugate is added. During the incubation, a complex is generated either among antigen coated microparticles, the Anti-HIV within the sample and enzyme-labeled antigen or antibody coated microparticles, p24 antigen in the sample, biotin p24 antibody and HRP labeled streptavidin by immunological reactions. After washing, the substrates are 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 HIV antibody/antigen in the samples.

Materials provided

1. Positive Control 1

1 vial containing 1.0 mL of Tris-HCl buffer, containing heat-inactivated human serum or plasma positive for anti-HIV-1 and proteins of bovine origin and ProClin 300® preservative. The plasma is nonreactive for anti-HIV-2, HBsAg, anti-HCV and syphilis. Reagent provided ready to use.

2. Positive Control 2

1 vial containing 1.0 mL of Tris-HCl buffer, containing HIV p24 antigen and proteins of bovine origin and ProClin 300® preservative

3. Negative Control

1 vial containing 1.0 mL of PBS buffer, containing proteins of bovine origin. Contains ProClin 300® preservative. Reagent provided ready to use.

The negative control is nonreactive for HBsAg, HIV-1 and HIV-2 antibodies and antigens, anti-HCV and syphilis.

4. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3mL*2	2.3mL*5	1.2mL*2
Enzyme Conjugate	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2
Biotin p24 Antibody Solution	2.5mL*1	4.0mL*1	4.0mL*2	4.0mL*5	2.5mL*2

- Microparticles Solution

Contains HIV-1(Group M and Group O)/HIV-2 antigen and HIV p24 antibody coated microparticles in Tris-HCl buffer containing BSA. Contains ProClin 300® preservative.

- Enzyme Conjugate

Contains horseradish-peroxidase labeled HIV-1(Group M and Group O)/HIV-2 antigen and streptavidin in Tris-HCl buffer containing proteins of bovine origin. Contains ProClin 300® preservative.

- Biotin p24 Antibody Solution

Contains biotin p24 antibody in Tris-HCl buffer containing proteins of bovine origin. Contains ProClin 300® preservative.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

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

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample cup(s) or tube(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. This assay may contain 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. 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.
10. Do not use reagents beyond the labeled expiry date.
11. Do not mix or use components from kits with different batch codes.
12. When storing the controls, be certain the vials are securely sealed.
13. Ensure the microparticles are resuspended before loading it on the analyzer.
14. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
15. Do not substitute any reagent in this kit from other manufacturers or other lots.
16. 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 unsealed reagents pack upright on the analyzer or 2-10℃ 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 2-10 ℃ in an upright position.
4. Seal and return the remaining Positive Controls and Negative Controls at 2-8℃ immediately after the experiment, under which conditions the stability will be retained for 1 months.

Sample

1. Do not use samples with the following conditions:
   - heat-inactivated
   - pooled
   - grossly hemolyzed (>1 g/L)
   - obvious microbial contamination
   - cadaver samples or any other body fluids
   - sodium azide preservative
2. Collect samples in accordance with correct medical practices.
3. Ensure complete clot formation in serum samples before centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time.
4. Samples from heparinized patients may be partially coagulated and contain fibrin. Draw the sample prior to heparin therapy.
5. For accurate results, serum and plasma samples should be free of fibrin, red blood cells or other particulate matter.
6. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
7. For optimal results, inspect all samples for bubbles. Remove bubbles with a pipette tip prior to analysis. Use a new tip for each sample to prevent cross contamination.
8. Samples must be separated from clots or red blood cells using centrifugation as recommended by the tube manufacturer. Gravity separation is not sufficient for sample preparation.
9. To ensure consistency in results, samples containing particulate matter or red blood cells, samples that have been thawed, and samples that require retesting must be transferred to a centrifuge tube and centrifuged at > 10,000 RCF (Relative Centrifugal Force) for 10 minutes prior to testing.
10. Mix thawed samples by inverting 10 times. Visually inspect the samples for the absence of stratification. If layering or stratification is observed repeat inversion cycles until samples are visibly homogeneous. Centrifuge prior to testing.
11. Centrifuged samples with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified sample without the lipemic material.
12. 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.
13. Cap and store the samples at 18-30 ℃ for no more than 8 hours, for longer use, freeze the samples that need to be stored or transported at -20 ℃. Avoid multiple freeze-thaw cycles.
14. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
15. 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 cups or tubes on the sample rack, 100 μL of sample and controls 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 rack 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 Biotin p24 antibody solution 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 HIV antibody/antigen 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 positive controls and negative control into the sample cups or tubes and place them on the sample rack. 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 instrument failure.
- Load the sample rack and input negative & positive control information on the system software interface.
- Select "run" to start the test and generate the calibration which 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.

Results

- Calculation

The Assay Analyzer system calculates the Cut-off value using the mean chemiluminescent signal (RLU) from the three replicates of the Positive Control 1 and stores the result.

This assay calculates results based on a cut-off value determined by the following calculation:

1. Cut-off Values = Positive Control 1 mean RLU Value x 0.4
2. S/CO = Sample RLU/Cut-off Value
3. Assay analyzer calculates a result based on the samples RLU to the cut-off value for each sample and control.

- Interpretation of Results

Samples with S/CO values <1.00 are considered nonreactive (NR).

Samples with S/CO values $\geqslant 1.00$ are considered reactive (R).

Note: All samples that are initially reactive must be centrifuged and retested in duplicate.

HIV Ag/Ab Combo CLIA Microparticles results

Initial Result	Retest Results (S/CO)	Final Result	Interpretation
R	Both tests are NR	NR	HIV p24 Ag and/or HIV-1/HIV-2 Ab not detected
R	One or both are reactive	R	Presumptive evidence of HIV p24 Ag and/or HIV-1/HIV-2; perform a supplemental assay
NR	No retest required	NR	HIV p24 Ag and/or HIV-1/HIV-2 Ab not detected

The Interpretation of Results for samples with a final result of reactive by this HIV Ag/Ab Combo assay and indeterminate by supplemental testing is unclear; further clarification may be obtained by testing another sample taken three to six weeks later.

The HIV Ag/Ab Combo and supplemental assay results should be interpreted in conjunction with the patient's clinical presentation, history, and other laboratory results.

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 following assigned specifications for the controls are met:

1. Mean PC 1 RLU/ Mean NC RLU>10
2. Mean PC 2 RLU/ Cut-off value >1 (only when Positive Control 2 is tested)

When the controls fail 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. A test result that is nonreactive does not exclude the possibility of exposure to or infection with HIV-1 and/or HIV-2. Nonreactive results in this assay for individuals with prior exposure to HIV-1 and/or HIV-2 may be due to antigen and antibody levels that are below the limit of detection of this assay.
6. 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.
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.

Performance Characteristics

1. Measurement Precision

This within-run precision is based on 3 internal controls (1-high, 2-medium and 3-low) were assayed, using 3 batches of reagents, in replicates of 20. Data from one batch are summarized in the following table.

Panel Member	Batch	n	Mean	Within-run Precision	
				SD	%CV
1	1	20	9.78	0.33	3.41
2	1	20	5.51	0.25	4.46
3	1	20	2.87	0.13	4.54

This between-day precision is based on 3 internal controls (1-high, 2-medium and 3-low) tested in 2 runs per day for 20 days on 3 batches of reagents. Data from one batch are summarized in the following table.

Samples	Lot	n	Between-day Precision	
			Mean	%CV
1	1	40	13.47	6.41
2	1	40	7.00	4.30
3	1	40	3.04	5.49

*Representative data; results in individual laboratories may vary from these data.

2. Sensitivity

In total 1082 HIV positive samples were tested with the HIV Ag/Ab Combo CLIA Microparticles. All samples obtained positive test results. The sensitivity of HIV positive samples is 100%.

3. Specificity

A total of 9307 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. Of the 9307 samples, 10 (9297/9307) samples were repeatedly reactive, were negative based on supplemental test results from a licensed and/ or research immunoblot assay. The specificity of this assay is 99.89%.

- Analysis specificity

Cross reaction: this assay was evaluated for potential cross-reactivity for 62 samples from individuals with medical conditions unrelated to HIV infection. The samples were tested using the HIV Ag/Ab Combo CLIA Microparticles assay. The data are summarized in the following table.

Category	No.	HIV Ag/Ab Combo CLIA Microparticles assay	
		Reactive	Nonreactive
CMV IgG	3	0	3
CMV IgM	3	0	3
ANA	3	0	3
RF	3	0	3
EBV	3	0	3
HCV	3	0	3
TP	3	0	3
HAV	3	0	3
HEV	3	0	3
Rubella IgG	3	0	3
Rubella IgM	3	0	3
HSV-1 IgG	3	0	3
HSV-1 IgM	3	0	3
HSV-2 IgG	3	0	3
HSV-2 IgM	3	0	3
Toxo IgG	3	0	3
Toxo IgM	3	0	3
HBV	11	0	11

a. Rh.[a] factor: Rheumatoid factor

The results of 62 samples evaluated above have no cross-reactivity with the test.

Interference: at the concentrations listed below, bilirubin (conjugated and unconjugated), haemoglobin and triglycerides showed less than 10% interference in the HIV Ag/Ab Combo CLIA Microparticles assay. All tests were performed in triplicate. According to the results, at the concentrations listed below, the tested substances have no interference.

- Bilirubin ≤0.4g/L
- Haemoglobin ≤1g/L
- Triglycerides ≤50g/L

- 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 non-infected individuals and were tested in the HIV Ag/Ab Combo CLIA Microparticles assay. The results showed no statistically significant difference between serum and plasma. HIV Ag/Ab Combo CLIA Microparticles assay is suitable for testing serum and plasma samples with EDTA, heparin or sodium citrate plasma.

Literature References

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