Coulter HIV p24

1. PRINCIPLE

The Human Immunodeficiency Virus Type 1 (HIV-1) is recognized as the etiologic agent of acquired immunodeficiency syndrome (AIDS). The virus is transmitted by sexual contact, exposure to infected body fluids or tissues, and from mother to fetus or child during perinatal period. After exposure to the virus, HIV-1 infection is characterized by an early period of antigenemia in which HIV-1 antigens (Ag) are detectable in blood. In most individuals, the antigen level becomes undetectable for a period of time; late in disease, increasing failure of the immune system and increasing levels of virus may again result in detectable levels of antigen. One of the viral components in blood during antigenemia is the core protein, p24, the major internal structural protein of HIV-1.

The Coulter HIV-1 p24 Antigen Assay is an enzyme immunoassay (EIA, or Enzyme-linked Immunosorbant Assay, ELISA) developed for detection and quantitation of the HIV-1 p24 core protein. The Coulter HIV-1 p24 Antigen Assay uses a murine monoclonal antibody to HIV-1 p24 antigen coated onto microtiter strip wells. A specimen of plasma, serum or tissue culture media and lysis buffer are added to a coated well and incubated. If present, the virus antigen particles bind to the monoclonal antibody on the microtiter well. Following a wash step, biotinylated human anti-HIV-1 IgG is added to the well which during incubation, binds to any HIV-1 p24 antigen bound to the well. Following another wash step, streptavidin-horseradish peroxidase is added which complexes with biotinylated antibodies. In a final step, a substrate reagent containing tetramethylbenzidine (TMB) and hydrogen peroxide is added which reacts with complexed peroxidase to form a blue color. The reaction is terminated by the addition of acid, and the absorbance is measured spectrophotometrically. The intensity of the color development is directly proportional to the amount of uncomplexed p24 antigen in the plasma, serum or tissue culture media. The quantity of free HIV-1 p24 antigen in a specimen is determined by comparing its absorbance with that of known HIV-1 p24 antigen standard curve.

2. SPECIMEN REQUIREMENTS

2.1 Serum, tissue culture supernatant, or plasma collected in acid-citrate-dextrose (ACD), citrate-phosphate-dextrose with adenine (CPDA-1), EDTA, sodium citrate or heparin may be used and should be tested as soon as possible following collection. If the situation limits the ability to test the sample quickly, the specimen can be held in refrigeration (2-4° C) for a maximum of 7 days. If the period of time will be greater, the sample can be held at -20°C or -85°C for long-term storage.

2.2 Remove the serum from the clot or plasma from the red cells as soon as possible to avoid hemolysis.

2.3 Heat-inactivated specimens or specimens with obvious microbial contamination are unacceptable.

2.4 Specimens containing particulate matter may give inconsistent results. Such specimens should be clarified by centrifugation prior to assay.

2.5 Avoid subjecting specimens to repeated freeze thaw cycles.

2.6 Bring all specimens to room temperature (15-30°C) prior to assay.

3. REAGENTS

3.1 Reagents included in Coulter HIV-1 p24 Antigen Assay Kit, 96 (PN 6604534) or 2400 (PN 6607051), include the following:

3.1.1 HIV p24 Antibody-coated Microtiter Strips. Store at 2-8°C. Note manufacturer's outdate.

- Bring pouch containing HIV-1 p24 antibody coated microtiter strips to room temperature (15-30°C) before opening to avoid condensation on the strips.
- The plate consists of 12 removable strips of 8 wells each. Any partial use of strips commits all 8 wells to the assay. Antibody coated strips may be used only once. When using a 96 well plate washer and fewer than 12 strips are needed, place uncoated strips in the remaining positions.
- Unused strips may be placed back into the pouch and sealed with the desiccant provided and stored at 2-8°C for 60 days.

3.1.2 Anti-HIV (human) Biotin Reagent. Store at 2-8°C. Note manufacturer's outdate. The reconstituted reagent is stable for 2 months. Bring to room temperature (15-30°C) prior to assay.

- Add 21 mL of distilled water to the Biotin Reagent vial and recap the vial.
- Gently invert vial to mix contents. Allow 5 minutes for the contents to dissolve.

3.1.3 Normal Human Serum (NHS). Store at 2-8°C. Note manufacturer's outdate.

3.1.4 SA-Buffer (Tris buffer for SA-HRPO). Store at 2-8°C. Note manufacturer's outdate.

3.1.5 SA-HRPO (Streptavidin conjugated to horseradish peroxidase). Store at 2-8°C. Note manufacturer's outdate. Prepare SA-HRPO Working Dilution as follows.

- Within 15 minutes prior to use, prepare the SA-HRPO Working Dilution. For a complete 96 well plate, add 21 μL of SA-HRPO reagent to 21 mL of SA-HRPO Buffer. Mix well and use.
- If a partial plate is used, prepare enough SA-HRPO Working Dilution as shown below.

No of Tests	SA Buffer (mL)	SA-HRPO (μL)
24	5.0	5
48	10.0	10
72	15.0	15

- Discard unused portion at the end of the day.

3.1.6 TMB Diluent. Store at 2-8°C. Note manufacturer's outdate.

3.1.7 TMB Reagent in Dimethyl sulfoxide. Store at 2-8°C. Note manufacturer's outdate. Prepare TMB-substrate Solution as follows:

- Within 15 minutes prior to use, prepare the TMB-Substrate Solution. For a complete 96 well plate add 21mL to the TMB Diluent into a clean disposable plastic container and add 210 μL of TMB Reagent. Mix well and use.
- If a partial plate is used, prepare enough TMB-Substrate as follows:

No of Tests	TMB Diluent (mL)	TMB Reagent (μL)
24	5.0	50
48	10.0	100
72	15.0	150

- Discard unused portion at the end of the day.
- Note: TMB-Substrate Solution should appear colorless and, when combined with CSR-1 Solution, should have an absorbance value less than 0.050 at 450 nm 450/570 nm when compared wit a distilled water blank.

3.1.8 Lysis Buffer. Store at 2-8°C. Note manufacturer's outdate.

3.1.9 Wash Buffer. Store at 2-8°C. Note manufacturer's outdate. Prepare working Wash Buffer as follows:

- To prepare 700 mL of Wash Buffer, dilute 35 mL of 20X Wash Buffer with 665 mL of distilled water.
- Discard any unused portion at the end of the day.

3.1.10 Coulter Stop Reagents-1 (CSR-1) (4N H2SO4). Store at 2-30°C. Note manufacturer's outdate.

3.2 Reagents required but not provided:

3.2.1 5% Hypochlorite solution (household bleach) diluted 1/100 or appropriate disinfectant.

3.2.2 Dionized or distilled water.

3.2.3 Standards and controls for the assay provided by the Virology Quality Assurance Laboratory (VQA):

3.2.3.1 VQA SQC (Serum Quality Control). A set of five concentrations. Store at -80°C.

- Just prior to set up, thaw 1 vial of each of the 5 concentrations.
- Mix well and use.

3.2.3.2 VQA 400pg/mL standard, diluent and Media Correction Control (MCC) for culture supernatants

- Prepare 2 fold serial dilutions from 400pg/mL Standards using the VQA diluent.
- Run 0 (diluent), 12.5, 25, 50 and 100pg/mL standard curve in duplicate, run MCC (30pg/mL) in duplicate

4. SUPPLIES AND EQUIPMENT

4.1 Lab coat

4.2 Gloves

4.3 Micropipette(s) capable of delivering 10 μL, 20 μL, 50 μL, 200 μL volumes

4.4 Multichannel pipette(s) capable of delivering 10 μL, 20 μL, 50 μL, 200 μL volumes

4.5 Disposable pipette tips suitable for the above pipettes

4.6 Disposable reagent reservoirs

4.7 Uncoated 96 well microtiter plate

4.8 Incubator without CO2 capable of maintaining 37°C +/- 1°C

4.9 Timer capable of measuring times up to 60 minutes

4.10 Centrifuge

4.11 Graduated cylinders and beakers

4.12 Serological pipettes

4.13 ELISA microtiter plate washer with waste trap and vacuum source

4.14 ELISA microtiter plate reader capable of measuring absorbance at 450 nm with reference at 570 nm

5. PROCEDURE

5.1 Plate Set-up

5.1.1 Bring all reagents and samples to room temperature.

5.1.2 Create an EIA template in the virology data-management software (see software manual).

5.1.3 Position the required number of microtiter strips in the strip holder reaction plate (8 wells per strip). If fewer than 12 strips are needed, use uncoated strip(s) in the remaining positions when using a 96 well plate washer.

5.1.4 Add 20 μL of Lysis Buffer to each test well of the coated microtiter plate.

5.1.5 Add 200 μL of each VQA SQC concentration and each specimen to the coated microtiter plate according to the template. Cover the plate using an adhesive plate cover.

5.1.6 Incubate at 37°C for 1 hour + 5 minutes.

5.1.7 Wash as follows: Aspirate the solution from the wells. Add 300 µL pf Wash Buffer Working Dilution to each well. Allow wells to soak for 25-30 seconds. Aspirate the solution from the wells. Wash five (5) more times for a total of 6 washes. After the final wash step, grasp the plate firmly along the edges, invert plate over absorbent paper and tap the plate gently to remove any remaining liquid.

IMPORTANT: The time between the wash step and the next reagent must be less than five (5) minutes.

5.1.8 Add 200 µL of reconstituted Biotin Reagent to all testing wells, except the substrate blank well. Cover the plate using a new adhesive paper cover. Incubate at 37°C + 2 for 1 hour + 5 minutes.

5.1.9 Wash as described above.

5.1.10 Add 200 µL of SA-HRPO Working Dilution to all testing wells, except the substrate blank well. Cover the plate using new adhesive plate cover. Incubate at 37°C + 2 for 30 + 2 minutes.

5.1.11 Wash as described above.

5.1.12 Add 200 µL of TMB-Substrate Solution to all wells. Cover the plate using a new adhesive plate cover. Incubate at room temperature (15-30°C) for 30 + 2 minutes.

5.1.13 Add 50 µL of CSR-1 to all wells.

IMPORTANT: Add CSR-1 to the wells in the same sequence and at the same rate of speed that the TMB-Substrate Solution was added.

5.1.14 Read absorbance at 450 nm (reference at 570 nm if dual wavelength Instrument is available) within 30 minutes of adding CSR-1 to the wells.

6. CALCULATIONS

The HIV-1 p24 antigen concentrations may be generated from the LDMS. A weighted linear least squares method using the VQA SQC or MCC-corrected concentrations is used to estimate HIV-1 p24 antigen concentration for serum or culture samples.

7. QUALITY CONTROL

The OD values obtained from the spectrophotometer may be transferred into the LDMS directly or indirectly using the remote read software. A run report may be generated

that includes the raw OD values and calculated p24 results in pg/mL for each sample in the run. Assay validity must be determined by comparing the obtained OD values for each control and standard to an established range. Acceptable OD values must be established within each laboratory for each lot of VQA controls. Kit controls may also be included in each run and should satisfy the criteria outlined in the manufacturer's package insert. The run should be reviewed by the laboratory manager or director prior to data release. The laboratory director/manager must determine the significance of any out of range QC and resolve the situation prior to releasing any results.

8. PROCEDURAL NOTES

The incubation at 37°C is critical. If the temperature goes above 38°C, coagulation of the samples may occur.

If a sample gels completely and the well still contains visible coagulated serum proteins after washing, the results should be considered invalid and the sample retested.

9. REFERENCES

Coulter HIV-1 p24 Antigen Assay package insert and all references within.

Procedure: ACTG Lab Man Coulter HIV1 p24 ELISA

Prepared by: ACTG Laboratory Technologist Committee

Preparation Date: 01 June 2004

Date Implemented into the Laboratory: _____

Updated on:

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Reviewed by: Date:

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Supersedes Archived Protocol: DAIDS Virology Manual for HIV Laboratories, Version January 1997