Perklin Elmer/NEN Life Science Products HIV-1 p24 ELISA

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. Later in disease progression, an 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 NEN HIV-1 p24 ELISA is an enzyme immunoassay (Enzyme-Linked Immunoabsorbant Assay) developed for detection and quantitation of the HIV-1 p24 core protein. The NEN HIV-1 p24 ELISA utilizes a mouse monoclonal antibody to HIV-1 p24 antigen, which is coated onto micro-titer strip wells. A neutralized specimen of plasma, serum or tissue culture supernatant are added to a coated well and incubated. If present, the virus antigen particles bind to the monoclonal antibody on the micro-titer well. Following a wash step, biotinylated human polyclonal antibody to HIV-1 p24 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 conjucate is added which complexes with biotinylated antibodies. In a final step, a substrate reagent containing orthophenylenediamine-HCL (OPD) is added which reacts with complexed peroxidase to form a yellow 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 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 All biologic samples and culture supernatants should be inactivated prior to being tested (i.e. 50ul 5% Triton X-100 plus 450uL sample, vortex well). If samples are not inactivated before setting up the assay, then you must work in a bio-safety cabinet while adding reagents to the plate.

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

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

2.6 Avoid subjecting specimens to repeated freeze thaw cycles.

3. REAGENTS

3.1 Perkin Elmer/NEN HIV-1 ELISA Kit

- Catalog numbers include NEK050 (One 96-well plate), NEK050A (Two 96 well plates), and NEK050B (Five 96 well plates). Currently the kit is available from Perkin Elmer.
- Do not use reagents beyond the kit expiration date
- Use only the reagent lots assigned to the kit.

3.2 Reagents Included in the Kit:

3.2.1 Antibody-coated Microplate

Store at 2-8°C. To avoid condensation, bring the pouch containing the HIV-1 p24 antibody coated microplate to room temperature (15-30°C) before opening. 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.

3.2.2 5% Triton X-100

Store at 2-8°C. Bring to room temperature (15-30°C) prior to assay.

3.2.3 Detector Antibody (Rabbit polyclonal anti-p24 antibody)

Store at 2-8°C. Bring to room temperature (15-30°C) prior to assay.

3.2.4 Streptavidin-HRP Diluent

Store at 2-8°C. Bring to room temperature (15-30°C) prior to assay.

3.2.5 Streptavidin-HRP Concentrate

Store at 2-8°C. Bring to room temperature (15-30°C) prior to assay. Within 15 minutes of use, prepare Streptavidin-HRP by making a 1:100 dilution of Streptavidin-HRP Concentrate with Streptavidin-HRP Diluent. To prepare the working concentration for a complete 96 well plate add 120uL of the Streptavidin-HRP Concentrate to 12mL of Streptavidin-HRP Diluent. Protect working solution from light. If a partial plate is used, prepare enough Streptavidin-HRP working concentration for the number of rows used. As a guideline, 1ml of Streptavidin-HRP solution is sufficient for one strip of wells.

3.2.6 Substrate Dilent

Store at 2-8°C. Bring to room temperature (15-30°C) prior to assay.

3.2.7 OPD Tablets

Store at 2-8°C. Bring to room temperature (15-30°C) prior to assay. Within 15 minutes of use, prepare sufficient OPD Substrate Solution. With non-metallic forceps or the equivalent, add 1 OPD tablet to 11mL of Substrate Diluent. This is enough OPD for one assay plate. Vortex vigorously to assure that the tablet goes into solution completely. Protect from light. The OPD substrate solution should be colorless or very pale yellow. A yellow-orange color indicates deterioration and the solution should not be used. OPD is toxic by inhalation, in contact with skin and if swallowed. It is also a possible cancer hazard. If skin is contacted, flush with water. Solutions containing OPD should be disposed of according to local regulations.

3.2.8 Plate Wash Concentrate, 20x

Store at room temperature (15-30°C). Diluted (1x) wash buffer should be prepared fresh prior to use. Dilute Plate Wash Concentrate 20x by adding 1 part concentrate to 19 parts distilled, deionized water (i.e., 100mL Wash Concentrate/1900ml water). Approximately 1000mL of diluted (1x) wash buffer is needed per plate assayed. More or less may be needed depending on the type of washer used.

3.2.9 Stop Solution (4N sulfuric acid)

Store at room temperature (15-30°C).

3.3 Additional Reagents Required (Not available in Kit)

3.3.1 Hypochlorite solution (household bleach)

Diluted 1/100 or appropriate disinfectant.

3.3.2 Distilled, dionized water

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

3.3.3.1 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.

3.3.3.2 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 10-1000 μL volumes

4.4 Multichannel pipette(s) capable of delivering 100 μL volumes

4.5 Disposable pipette tips suitable for the above pipettes

4.6 Disposable reagent reservoirs

4.7 Incubator capable of maintaining 37°C +/- 1°C

4.8 Timer capable of measuring times up to 2 hours

4.9 Centrifuge

4.10 Graduated cylinders and beakers

4.11 Serological pipettes

4.12 Uncoated microplates

4.13 ELISA microtiter plate washer with waste trap and vacuum source

4.14 ELISA microtiter plate reader capable of measuring absorbance at 490 nm or 492 nm and > 600nm filter capability.

5. PROCEDURE

5.1 Inactivate Samples

For safety reasons, it is advisable to inactivate all samples before testing with the NEN HIV-1 p24 ELISA kit. This will also allow you to work outside of the bio-safety cabinet. To inactivate the samples add 50ul of 5% Triton X-100 to 450uL of sample. Vortex well.

5.2 Plate Set-up

5.2.1 Bring all reagents and samples to room temperature.

5.2.2 Create a plate template in the assay module of LDMS. See the LDMS users manual for specific instructions, found at http://www.fstrf.org/ldms/ldms.html.

5.2.3 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.

5.2.4 VQA standards and control wells: Add 20 μL of 5% Triton X-100 and 200ul of the standard/diluent to the appropriate well.

5.2.5 Sample wells: If samples were inactivated already, then add 220uLof sample to the appropriate well. If samples were not inactivated yet, then add 20uL of 5% Triton X-100 and 200uL of sample to the appropriate well.

5.2.6 Incubate at 37°C for 2 hours ± 5 minutes.

5.2.7 Wash plate six times with 300 μL/well of diluted (1x) wash buffer. Plate washing may be automated, semi-automated or manual but must be carried out with care to ensure optimal assay performance. 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.

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

5.3 Detector Antibody

5.3.1 Add 100 μL of Detector Antibody to all wells, except the substrate blank well. Cover the plate using a new adhesive paper cover. Incubate for 60 ± 5 minutes at 37°C.

5.3.2 Wash the plate as described above.

5.4 Streptavidin-HRP

5.4.1 Add 100 μL Streptavidin-HRP Working Solution to all testing wells, except the substrate blank well. Cover the plate using new adhesive paper cover. Incubate at room temperature (15-30°C), in the dark, for 30 ± 5 minutes.

5.4.2 Wash the plate as described above.

5.5 OPD Substrate Solution

Add 100 μL of freshly prepared OPD-Substrate Solution to all wells. Cover the plate using new adhesive paper cover. Incubate at room temperature (15-30°C), in the dark, for 30 ± 5 minutes.

5.6 Stop/Read Plate

5.6.1 Add 100 μL of Stop Solution to all wells.

5.6.2 Read absorbance at 490 or 492 nm. Readings must be taken with a reference filter at >600 nm. The plate should be read within 15 minutes after stopping the reaction. Be sure the bottom of the plate is clean and dry prior to reading.

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. Prior to releasing the data, the run should be reviewed by the laboratory manager or director. 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

Addition of reagents must be in the order specified. Reagents and samples must be added to the plate in a timely manner.

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

NEN HIV-1 p24 ELISA package insert and all references within.

Procedure: ACTG Lab Man Perkin Elmer HIV-1 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