

STANDARD OPERATING PROCEDURE

(Name of the Blood Centre)

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LOCATION	SUBJECT
TTI Testing Laboratory	Anti HIV Testing
FUNCTION	DISTRIBUTION
Sample tested for Anti HIV antibodies by EIA	- Supervisor in charge of TTI Testing Laboratory - Master File

1. SCOPE & APPLICATION

Anti HIV antibodies testing is carried out on all bag samples before these are released for transfusion. Pre-donation samples of pheresis donors are also tested.

2. RESPONSIBILITY

It is the responsibility of technician from TTI Testing lab; to carry out the test and report as required.

3. REFERENCE

- Kit package insert.
- Technical Manual of American Association of Blood Banks 13th Edition, 1999. Pages 152-153.

4. MATERIALS REQUIRED

- Reagent kit
- Micropipettes and disposable pipette tips
- Timer
- EIA reader
- EIA Washer
- Incubator 37°C
- Vortex Mixer
- Glassware
- Distilled water.

5. PROCEDURE

5.1 Principle

Human serum or plasma diluted in specimen diluent and incubated with the proteins of HIV 1 HIV 2, coated auto microplate wells and incubated. If the HIV antibodies are present in the sample that are tested, it will bind with the proteins coated on the microwell. After washing off the unbound analyte, horse radish peroxidase conjugated with anti human IgG antibodies is added. Enzyme conjugate binds through the antigen antibody complex if present. Unbound analyte is washed and substrate solution is added. Colour will develop in proportion to the amount of HIV antibodies present in the specimen. Stopping solution is added at the end of the incubation to stop the reaction. The reaction is read by EIA reader.

5.2 Method

The anti HIV antibody test is carried out as per the instructions given in the package insert of the kit.

1. Remove reagents from the fridge 30 minutes prior to testing. Mix the reagents gently by inverting the vials without foaming.
2. Bring the samples to room temperature before testing.
3. Arrange all samples to be tested serially in ascending order in which they are to be tested in a test tube rack.
4. Place the plate in front of the test tube rack
 - Add 100 μ l of the sample diluent to A-1 well as blank
 - Add 100 μ l negative control in each well No. B-1, C-1 respectively.
 - Add 100 μ l positive control in D-1, E-1 and F-1 wells
 - Add 100 μ l of each sample diluted in sample diluent (1:11) in each well, starting from G1 well.
 - Apply cover seal
 - Incubate at 37°C \pm 2°C for 30 min. \pm 2 min.
 - While the samples are incubating, prepare working wash solution and working conjugate as specified in package insert.
 - Take out the plate from the incubator after the incubation time is over and wash the wells 5 times with working wash solution.
 - Add 100 μ l of working conjugate solution in each well including A-1.
 - Apply cover seal
 - Incubate at 37°C \pm 2°C for 30 min. \pm 2 min.
 - Aspirate and wash as described in step No. (h).
 - Add 100 μ l of working substrate solution in each well including A-1.
 - Incubate at room temperature (20-30°C) for 30 min. in dark.
 - Add 50 μ l of stop solution.
 - Read absorbance at 450 nm within 30 minutes in Elisa Reader after blanking A-1 well.

Printer prints out all nos. fed in and their OD values. Reactive is printed across that particular reactive sample number according to the O.D. value of the cutoff.

5.3 Validation:

- Check the validity of the Blank (if used) as well as negative and positive control absorbance value as per pack insert of the kit.
- Examine absorbance values of the controls before the sample results can be interpreted. If the run fails to meet the criteria as per package insert consider the test as invalid and repeat the whole test again.
- Cut off O.D. is automatically calculated.

5.4 Interpretation:

Check the printout carefully for absorbance values:

- (i) The samples below the cut off are considered non-reactive.
- (ii) Equal to cut off are considered initially reactive (I.R)
- (iii) Above cut off are considered I.R.
- (iv) Sample close to cut off value 10% below cut off (grey zone)

Samples with grey zone results are repeated in one well.

6. DOCUMENTATION

6.1

Paste the print out in the HIV register and also record the following details:

- (a) The date on which the test is run.
- (b) The name of the kit used.
- (c) Lot No and expiry date of the kit.
- (d) Initials of the Technologist who performed the test and the Supervisor who verified the results.
- (e) The reactive units are marked in red.

6.2

Transfer the record to donor records.

7. END OF DOCUMENT