**Model SOP**

**Standard Operating Procedure**

**Name of the facility / activity : Anti HIV Testing of Blood unit (by Elisa**

**Method)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **SOP no.** | **Effective Date** | **Pages** | **Prepared by** | **Authorised by** |
| 3.2.1 | 27-11-2000 | 5 |  |  |
| **Version** | **Review Period** | **Date of Review** | **Reviewed by** | **Number of copies** |
| VI | 2 years | 01-01-2015 |  | 10 |
| **LOCATION** : TTI Testing Laboratory | | | | |
| **SUBJECT** : Anti HIV Testing | | | | |
| **FUNCTION** : Sample tested for Anti HIV antibodies by ELISA method. | | | | |
| **DISTRIBUTION**: 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.

1. **RESPONSIBILITY:**

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

1. **MATERIALS REQUIRED:**

* Reagent kit
* Mircopipettes and disposable pipette tips
* Timer
* EIA reader
* EIA Washer
* Incubator 370C
* Vortex Mixer
* Glassware
* Distilled water.

**Specimen** – clotted blood sample of the donor.

1. **PROCEDURE**

**Preparation of the reagents**

1. Bring all the reagents to room temperature for 30 minutes before use.
2. Take the required number of strips from sealed HiV Antigen-antibody coated microplate, and the remaining strips must be kept at 2-80C with a silica gel (desiccant) in an aluminium pouch.
3. Preparation of Washing Solution

Make a 1:20 dilution of Washing Solution with distilled or de-ionized water (to the extent of required amount for example, add 10 ml of concentrated Washing Solution to 190 ml distilled or deionized water). Washing Solution may be crystallized at cool storage condition. If crystallized, use it after thawing at 370C water-bath maintained at 370C.

1. Preparation of Conjugate

Make a 1:51 dilution of Conjugate concentrate with Conjugate Diluent to the extent of required amount, 10 minutes before use (Refer Table 1. Shake well before use.

Table 1.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Strips  required | 1 | 2 | 4 | 6 | 8 | 10 | 12 |
| Conjugate diluents (ml) | 1 | 2 | 4 | 6 | 8 | 10 | 12 |
| Concentrated conjugate (µl) | 20 | 40 | 80 | 120 | 160 | 200 | 240 |

1. Preparation of Substrate

Make a 1:101 dilution of TMB Chromogen in DMSO with Substrate Buffer, 5 to 10 minutes before use (Refer Table 2). Avoid exposure to light. Substrate should be used only after thawing at 370C, if crystallized.

Table 2.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Strips  Required | 1 | 2 | 4 | 6 | 8 | 10 | 12 |
| Substrate Buffer (ml) | 1 | 2 | 4 | 6 | 8 | 10 | 12 |
| TMB Chromogen (µl) | 10 | 20 | 40 | 60 | 80 | 100 | 120 |

**STORAGE AND SHELF LIFE OF PREPARED REAGENTS**

|  |  |  |
| --- | --- | --- |
| **Prepared Reagent** | **Storage** | **Expiry date** |
| Wash Solution | 2-80C | 1 week |
| Substrate | Room temperature | 4 hours |

**ASSAY PROCEDURE**

1. Take the required numbers of strips and fix them to frame.
2. Pipette 100 µl of Negative Control into each well of 1A to 1B, 100 µl of antibody Positive Control into well of 1C, 100 µl of Antigen Positive Control into well 1D and pipette 100 µl of each sample into the remaining wells.

* **Take care not to mix or splash contents out of wells while using microplate shaker.**

1. Incubate at 37 ± 10 C for 60 minutes after sealing the plate with cover sealer.
2. Before the last 10 minutes of 1st incubation, make a 1:51 dilution of Conjugate with Conjugate Diluent.
3. Aspirate the contents from all the wells and wash each one 5 times with 300 µl of diluted Washing Solution. (300 µl/well/time)
4. Invert the plate and tap it on absorbent paper to remove the remaining Washing Solution, and then, pipette 100 µl of prepared diluted Conjugate into each well.
5. Incubate the plate at 37 ± 10 C for 30 minutes after sealing it with plate sealer.
6. Before the last 5 to 10 minutes of second incubation, make a 1:101 dilution of Substrate with Substrate Buffer.
7. Aspirate the contents from each of the wells and wash each one 5 times with 300 µl of diluted Washing Solution (300 µl/well/time)
8. Invert the plate and tap it on absorbent paper to remove the remaining Washing Solution. And then, pipette 100 µl of prepared substrate into each well and incubate at controlled room temperature (23 ± 20 C) for 30 minutes. Avoid exposure to light.
9. Pipette 100 µl of Stop Solution into each well and tap the plate gently to homogenize the colouring materials.
10. Read the absorbance at 450 nm (reference wavelength at 620 nm ) against an air blank within 30 minutes after addition of Stop Solution.

**QUALITY CONTROL**

* The average absorbance (PCx) of both the Positive Controls should be greater than or equal to 1.0.
* The average absorbance (NCx) of the Negative Control should be less than or equal to 0.100 and greater than – 0.005.

If the results are outside the above range, the test should be conducted again.

**INTERPRETATION OF RESULTS**

1. **Calculation of the cut off value.**
2. Calculation the Negative Control mean (NCx)

Negative control 1 absorbance = 0.045

Negative control 2 absorbance = 0.043

Negative control mean (NCx) = (0.045 + 0.043) / 2 = 0.044

* If 3 negative controls were tested, calculate a mean value of 3 Negative Controls.

1. Calculate the cut-off value

Cut off value = NCx + 0.200 = 0.044 + 0.200 = 0.244

1. **Interpretation**

Sample with absorbance greater that or equal to the cut off value are considered reactive to anti-HIV and HIV antigen. Samples with absorbance less than the cut off value are considered non reactive to anti-HIV and HIV antigen.

* **If the samples are considered reactive, the test should be conducted two more times.**

In case the re-tests show non reactive result the samples are considered negative, and on the other hand, If one of the re-tests shows reactive results the smaples are considered positive.

* **The sample considered positive shall be tested again by Western blot and etc. for final judgement.**

1. **DOCUMENTATION**

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

1. The date on which the test is run.
2. The name of the kit used.
3. Lot No and expiry date of the kit.
4. Initials of the Technologist who performed the test and the Supervisor who verified the results.
5. The reactive units are marked in red.

Transfer the record to donor records.

1. **REFERENCE:**
2. Kit package insert.
3. Technical Manual of American Association of Blood Banks – 15th Edition, 2005.
4. **END OF DOCUMENT**.