**Model SOP**

**Standard Operating Procedure**

**Name of the facility / activity : Sample Tested for HCV antibodies by ELISA**

**Method**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **SOP no.** | **Effective Date** | **Pages** | **Prepared by** | **Authorised by** |
| 3.3.1 | 27-11-2000 | 6 |  |  |
| **Version** | **Review Period** | **Date of Review** | **Reviewed by** | **Number of copies** |
| VI | 2 years | 01-01-2015 |  | 10 |
| **LOCATION** : TTI Testing Lab | | | | |
| **SUBJECT** : Anti HCV Testing | | | | |
| **FUNCTION** : Sample Tested for HCV antibodies by ELISA Method | | | | |
| **DISTRIBUTION**: Supervisor in charge of TTI Testing Laboratory  Master File | | | | |

1. **SCOPE & APPLICATION:**

Anti HCV 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. **Material Required:**

* Elisa Reader
* Elisa Washer
* Microshaker
* Incubator
* Micropipettes and disposable tips
* Timer
* Disposable gloves
* Disposal container with Na Hypochlorite
* Absorbant tissue
* Distilled water
* 1 mol / litre Sulphuric acid
* Kit

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 antigen 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) to make 200ml total volume and mix gently . 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 1:101 dilution of Conjugate concentrate with Conjugate Diluent 5 to 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) | 2 | 4 | 8 | 12 | 16 | 20 | 24 |
| Concentrated conjugate (µl) | 20 | 40 | 80 | 120 | 160 | 200 | 240 |

**Preparation of Substrate**

Make a 1:101 dilution of substrate 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) | 2 | 4 | 8 | 12 | 16 | 20 | 24 |
| TMB Chromogen (µl) | 20 | 40 | 80 | 120 | 160 | 200 | 240 |

Storage and expiry date of unsealed reagent

|  |  |  |
| --- | --- | --- |
| Prepared / Unsealed Reagent | Storage | Expiry Date |
| Antigen Coated Plate | 2-80 C | 1 month |
| Negative Control | 2-80 C | 1 month |
| Positive Control | 2-80 C | 1 month |
| Sample Diluent | 2-80 C | 1 month |
| Wash Solution | 2-80 C | 2 months |
| Conjugate Concentrate | 2-80 C | 1 month |
| Conjugate Diluent | 2-80 C | 1 month |
| TMB Chromogen in DMSO | 2-80 C | 1 month |
| Substrate Buffer | 20 C | 1 month |
| Stop Solution | 2-80 C or Room Temperature | Validity time of kit |

**STORAGE AND SHELF LIFE OF PREPARED REAGENTS**

|  |  |  |
| --- | --- | --- |
| **Prepared Reagent** | **Storage** | **Expiry date** |
| Wash Solution | 2-80C | 1 week |
| Substrate | 2-80C | 3 hours |
| Conjugate | 2-80C | 4 hours |

1. Take the required number of strips and fix them to plat.
2. Pipette 200 µl of Sample Diluent into each plate well and pipette 10 µl of Negative Control into each well from 1A to 1C and 10 µl of Positive Control into each well from 1D to 1E, respectively, and then, pipette 10 µl of each sample into the remaining wells. Mix it using microplate shaker at 1000 rpm for 10 seconds.

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

1. Incubate at 37± 10 Cfor 60 minutes after sealing the plate with cover sealer.
2. Before the last 5-10 minutes of 1st incubation, make a 1:101 dilution of conjugate with Conjugate Diluent.
3. Aspirate the contents from each of the wells and wash each well 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 200 µl of prepared diluted Conjugate into each well.
5. Incubate the plate at 37 ± 10 C for 30 minutes after sealing with cover sealer.
6. Before the last 5 to 10 minutes of 2nd incubation, make a 1:101 dilution of TMB Chromogen in DMSO 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 200 µl of prepared Substrate into each wwell. Incubate it at controlled room temperature (21-250 C) for 30 minutes. Avoid exposure to light.
9. Pipette 50 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 air within 30 minutes after pipetting the Stop Solution.

**QUALITY CONTORL**

* Absorbance of all the Positive Controls should be greater than or equal to 0.8.
* At least two of three Negative Controls should be greater than or equal to 0.000 and less than or equal to 0.200. Even the Negative Control mean (NCx) should be greater than or equal to 0.000 and less than or equal to 0.200.

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

**INTERPRETATION OF RESULTS**

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

Ex) Negative control 1 absorbance = 0.090

Negative control 2 absorbance = 0.085

Negative control 3 absorbance = 0.080

Negative control Mean (NCx) = (0.090 + 0.085 + 0.080) / 3 = 0.085

1. **Calculate the cut off value**

Cut off value = NCx + 0.3 =0.085 + 0.3 = 0.385

1. **Interpretation**

Samples with absorbance greater than or equal to the cut off value are considered positive to anti-HCV. Samples with absorbance less than the cut off value are considered negative to anti-HCV.

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

In case that the re-tests show negative, the samples are considered negative, and on the other hand, If one of the re-tests shows positive, the samples 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 HCV register and record the following details

* 1. The date on which the test is run.
  2. The name of the kit used.
  3. Lot number and expiry date of the kit.
  4. Initials of the Technologist who performs the test and Supervisor who verifies the results.
  5. Write the numbers of samples to be repeated below the print out. After these samples are repeated again then reactive or non-reactive is written across that unit below the print out.
  6. The reactive units are marked in red.

Transfer the record to donor records and grouping register.

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