**Model SOP**

**Standard Operating Procedure**

**Name of the facility / activity : Testing of Blood units for Hepatitis B Surface Antigen by ELISA method**

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| **SOP no.** | **Effective Date** | **Pages** | **Prepared by** | **Authorised by** |
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| **LOCATION** : TTI Testing Laboratory | | | | |
| **SUBJECT** : HBsAg Testing | | | | |
| **FUNCTION** : Samples tested for Hepatitis B Surface Antigen by ELISA method | | | | |
| **DISTRIBUTION**: Supervisor in charge of TTI Testing Laboratory  Master File | | | | |

1. **SCOPE & APPLICATION:**

HBsAg is a mandatory test for blood unit screened before it is transfused. This is carried out on all donor units’ samples. Anti-HBs is bound to the solid phase, the polystyrene microplate well. The test sample is incubated in the antibody-coated well. Through washing with buffer remove unreacted substances and leaves antigen if present attached to the surface of the well. A second antibody labelled with enzyme is then used to react with the trapped antigen. A second washing step removes unreacted enzyme- labelled antibody that is not bound to the antigen. The amount of enzyme left in the well is therefore proportional to the amount of antigen in the test specimen. The final step is testing for enzyme activity using the enzyme substrate. Depending on the enzyme substrate used, the assay may be read   
visually or quantitated by some optical system e.g. Spectrophotometer or ELISA reader.

1. **RESPONSIBILITY:**

It is the responsibility of technician from TTI Testing lab to carry out the test and report as required. The Medical Officer is responsible for cross checking all the test results and the entries in the register.

1. **MATERIALS 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
* Elisa Kit for HbsAg ( with microplate , reagents and controls)

**Specimen**

* Clotted blood / serum sample

1. **PROCEDURE:**

**Principle**

In the monoclonal EIA procedures microplate wells are coated with monoclonal antibody to Hepatitis B Surface Antigen (Anti HBs) are incubated with serum or plasma and Anti-HBs peroxidase (Horse radish) conjugate in one step assay. During the incubation period HBsAg if present is bound to the conjugate (Anti-HBs-HRPO). Unbound material is aspirated and washed away. On the addition of substrate colour develops in proportion to the amount of HBsAg which is bound. The enzyme reaction is stopped by the addition of stopping solution.

**Method**

General instructions:

1. **Carry out the test as per manufacturer’s instructions given in the package insert**.
2. Remove reagents from the fridge 30 minutes prior to testing. Mix the reagents gently by inverting the vials without foaming.
3. Bring reagents and samples to room temperature before testing.
4. Arrange all donor unit test tube samples, serially in ascending order in a test tube rack. Add required number of internal kit controls and external lab controls.
5. Discard all disposable tips into hypochlorite solution.
6. Place the tray in front of the test tube rack.

**PROCEDURE (Eliscan HBsAg)**

1. Fix the required numbers of strips to the frame.
2. Mix conjugate and conjugate diluents in the ratio 1:26.
3. Pipette 100 µl of negative control into each well of 1A to 1C and 100 µl of positive control into well of 1D and 1E, respectively, and then pipette 100 µl of each specimen into the remaining wells.
4. Pipette 25 µl of prepared conjugate ( of procedure 2) into each well and tap the frame gently to mix completely, and then incubate at 37± 10 C for 90 minutes.
5. Before the last 5 to 10 minutes (of the procedure 4) mix substrate solution and substrate buffer at the volume ratio of 1:101.
6. Aspirate the contents from all wells and wash each one 5 times with diluted washing solution (NLT 300 µl/well/time).
7. Invert the plate and tap it dry on absorbent paper. Pipette 100 µl of prepared substrate (of procedure 5) into each well and incubate at controlled room temperature (21-250 C) in dark for 30 minutes after mixing it with gentle tap.
8. Pipette 100 µl of stop solution into each well and shake well until the blue colour of each well turns into yellow colour.
9. Read the absorbance of positive, negative control and specimen at 450 nm (reference wavelength 620 nm) against air within 30 minutes.

**QUALITY CONTROL**

1. Negative control mean (NCx) should be greater than or equal to 0.000 and less than or equal to 0.200.
2. Positive control mean should be greater than or equal to 0.800.
3. If the results are outside the above range, the test should be repeated.

**INTERPRETATION OF RESULTS**

1. Calculation of the cut – off value
2. Calculate the negative control mean (NCx)

Ex negative control 1 absorbance 0.031

Negative control 2 absorbance 0.033

Negative control 3 absorbance 0.032 \* negative control mean (NCx)

= (0.031 + 0.033 + 0.032) / 3 = 0.032

1. Calculate the cut-off value

Cut – off value = NCx + 0.05 = 0.032 + 0.05 = 0.082

1. Interpretation

Sample with absorbance equal to or greater than the cut off value are considered reactive to HBsAg. Samples with absorbance less than the cut off value are considered non reactive to HBsAg.

**Calculation of results from reading by ELISA reader**

* Blank the instrument with air for 450/620 nm filter.
* Record the absorbance at 450/620 nm for each specimen and compute the mean   
  absorbance value for controls.
* The presence or absence of HbsAg is determined by relating the absorbance value of unknown samples to that of the cut-off value. The unknown samples whose   
  absorbance is greater than or equal to the cut-off value should be considered reactive for HbsAg. The unknown samples whose absorbance is less than the cut off value should be considered non-reactive. Ensure absolute cleanliness of the well bottom order to avoid abnormal absorbance values.

***Calculation for determining the cut-off value***

The cut-off value is obtained by adding the mean absorbance of the negative control   
values (NC) adding by 0.050.

Cut-off value = NC + 0.050

Note: Calculation of cut-off value may differ depending upon the kit and it is important to follow the manufacturer's instructions.

**Limitations**

Strict adherence to the instructions is necessary to obtain reliable results.   
Non-repeated positive results can occur due to technical errors such as:

* + - * Interchange of vial caps.
      * Use of the same tip for withdrawing different reagents or dispensing different samples.
      * Leaving the vials open for long.
      * Exposure of reagents to intense heat, light or strong sources of bacterial   
        contamination.
      * Inadequate rinsing of wells.
      * Contamination of well rims by conjugate or samples.
      * Use of reagents from different master lots.

**Validation:**

If the run fails to meet criteria as per package insert consider the test is invalid and repeat the whole test again. Examine absorbance values of the controls before the sample results can be interpreted.

Check the validity of the blank (if used) Negative and positive control absorbance value as per package insert of the kit.

Cut off O.D. is automatically calculated.

**Interpretation:**

Check the printout carefully for absorbance value:

1. The samples below the cut off are considered non-reactive.
2. Equal to cut off are considered initial reactive
3. Above cut off are considered initial reactive
4. Repeat all samples showing grey zone result.
5. **DOCUMENTATION:**

Paste the printout in the HBsAg file 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.
  5. Initials of the Supervisor who verifies the result.
  6. Reactive units are marked in red.

Transfer the results to TTI register and in case of reactive samples immediately issue instructions or make sure personally to remove the unit along with components.

1. **REFERENCE:**
2. Kit Package inserts.
3. Technical Manual of American Association of Blood Banks 15th edition 2005.
4. **END OF DOCUMENT.**