|  |
| --- |
| **STANDARD OPERATING PROCEDURE for Nigeria PreP Study** |
| **Study Site:**  | **SOPs Number** :LP-306 |
| **Title****HEPATITIS B SURFACE ANTIGEN (HBsAg) TEST** |
| **Version Number**:  | **Version Date:**  | **Effective date**:  |
| **Approval name Signature Date**  |

**Annual Review**

|  |  |  |
| --- | --- | --- |
| **Review date**  | **Revision Date**  | **Signature** |
|  |  |  |
|  |  |  |

**Document History**

|  |  |  |
| --- | --- | --- |
| **Version number**  | **Reason for change**  | **Date**  |
| 1.0 | Initial release  | 28th March 2015 |
|  |  |  |
|  |  |  |

**Distribution List**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name/Location**  | **No of copies** | **Name/Location**  | **No of copies** |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

1. **Introduction**

The detection of HBsAg in the serum/plasma indicates an infection caused by the hepatitis B virus.It is the first marker to appear and may be observed for 2-3 weeks before the clinical and biological symptoms of the disease. HBsAg persisiting beyond six months denotes chronic hepatitis .Thus,Monolisa HBsAg ULTRAassay is a one step enzyme immunoassy technique used for the dectection of the surface antigen Hepatitis B virus (HBsAg) in the human serum/plasma.

1. **Objectives**

This standard operating procedure (SOP) describes the procedure for carrying out HBsAg test in the study laboratory

1. **Responsibility**

The procedure cited is and will be performed by;

* All trained laboratory personnel on the serology bench
* All laboratory personnel certified okay after the training exercise by the trainer and head of the lab.
1. Abbreviations
* HBsAg: Hepatitis B surface antigen
* HBV: Hepatitis B virus
* EIA: Enzyme immunoassay
* NC: Negative control
* PC: Positive control
* Co: Cut Off
* LJ: Levey-Jennings
* EQA: External Quality Assessment
1. **Quality control**

All controls are included within each run (1 positive and 3 negative controls). The average absorbance values for each control are calculated. The values derived are plotted on the LJ chart after each run. At the end of each month, the chart is printed and pasted on the wall (see SOP for plotting LJ chart for detail).

**Lot to lot verification**

 A New kit lot/**shipment** is verified by using known HBsAg positive samples or residual panels from proficiency testing**.**

1. **Materials**

**Reagents**

|  |  |
| --- | --- |
| 1 | MICROPLATE: coated with monoclonal anti-HBs antibodies (mouse) |
| 2 | CONCENTRATED WASHING SOLUTION (20X)Tris NaCI buffer pH 7.4Preservative: proclin™300 (0.04%) |
| 3 | NEGATIVE CONTROLTris HCI buffer containing BSAPreservative: Proclin™ 300 (0.1%) |
| 4 | POSITIVE CONTROL (HUMAN)Tris HCI buffer containing BSA with addition of mixture of purified HBs Ag from various subtypesPreservative: ProClin™ 300 (0.1%) |
| 5 | CONJUGATE: Tris HCI buffer pH 7.4containing BSA, Tween 20 Preservative: ProClin™ 300 (0,1%), ciprofloxacine (10ug/ml) |
| 6 | CONJUGATE DILUENT: Mouse Monoclonal anti-HBs antibodies and GoatPolyclonal anti-HBs antibodies bound to the peroxidase, Lyophilized |
| 7 | SUBSTRATE BUFFERCitric acid and Sodium acetate solution pH 4.0Containing H2O2 (0.015% and DMSO (4%) |
| 8 | CHROMOGEN PINK COLOUREDSolution containing tetramethyl benzidine (TMB) |
| 9 | STOPPING SOLUTION1N sulphuric acid solution |

**Reagent preparation**

Concentrated washing solution

* Dilute 1:20 in distilled water to obtain the ready-for-use washing solution. Prepare 800 ml for one plate of 12 strips

Conjugate working solution

* Gently tap the vial of the lyophilized conjugate on the work-bench to remove any substance from the rubber cap.
* Carefully remove the cap and pour the content of a conjugate diluents vial into the lyophilized conjugate vial. Put the cap on and let stand for 10 minutes while gently shaking and inverting from time to time to ease dissolution

Enzyme development solution:

* Dilute 1:11 the chromogen in the substrate Buffer. Stability is for 6 hours in the dark once prepared.

|  |  |
| --- | --- |
| **Equipment** | **Supplies** |
| Precision pipettes & multichannel pipettes | Sodium hypochlorite (household bleach), Timer |
| Graduated cylinders, Micro plate incubator | Container for biohazard waste, Absorbent paper, Tips |
| Automatic microplate washer, Microplate reader | Distilled/deionized water, Disposable plate sealer |

**Equipment maintenance**

1. ELx50TM AUTOMATED STRIP WASHER DAILY MAINTENANCE

For all daily maintenance programs, ensure that the supply bottle contains at least 400ml of rinse solution, and that the waste bottle is empty.

Rinse

Daily maintenance involves flushing the washer with an appropriate reagent, usually deionized water, on the same day microplates are washed. This procedure helps prevent the aspirate and dispense tubes from clogging between washes or when left overnight or for the weekend. The DAY\_RINSE program is recommended for this process **in the morning and** the RINSE\_AND\_SOAK or OVERNIGHT\_LOOP program **is used at close of work**

To run the DAY\_RINSE program, follow the washer menu path shown below.

Select MAINT Press the option key until DAY\_RINSE appears

CONNECT RINSE AND PRESS <START> KEY MAINTENANCE RUNNING PRESS <STOP> TO QUIT.

Or , if the washer is equipped with automatic valve switching:

Select MAINT Press the option key until DAY\_RINSE appears RINSE VALVE? A B C START MAINTENANCE? YES MAINTENANCE RUNNING PRESS <STOP> TO QUIT.

Rinse and Soak

The RINSE\_AND\_SOAK program rinses the wash manifold and leaves the tubes soaking in the trough for the duration of the soak. See also Periodic Rinse and Soak.

To run the RINSE\_AND\_SOAK program, follow the washer menu shown below.

Select MAINT Press the option key until RINSE\_AND\_SOAK appears

CONNECT RINSE AND PRESS <START> KEY MAINTENANCE RUNNING PRESS <STOP> TO QUIT.

Or , if the washer is equipped with automatic valve switching:

Select MAINT Press the option key until RINSE\_AND\_SOAK appears CONNECT RINSE AND PRESS <START> KEY MAINTENANCE RUNNING PRESS <STOP> TO QUIT.

1. EMax® MICROPLATE READER.

A preventive maintenance plan is available below

**MAINTENANCE PLAN**

Equipment Service

|  |  |
| --- | --- |
| Incubator | Biannual |
| ELISA reader |  Biannual |
| ELISA washer |  Biannual |
| Pipettes | **Annual c**alibration |

**Reagent stability and storage**

|  |  |  |
| --- | --- | --- |
| Microplate (opened) | 2-8o C | 4 weeks |
| Diluted Wash buffer | 2– 30oC | **2 weeks** |
| Conjugate working solution (R6+R7) | 2– 8o C18-30oC | 4 weeks8 hours |
| Enzyme development solution | 18– 30oC(dark) | 6 hours |

**SAMPLE**

|  |  |  |
| --- | --- | --- |
| Sample type | Amount required | Storage/stabilities |
| Serum/plasma | 3ml | 2-8oc (<7days)-20oc (>7days) |

**SPECIAL SAFETY PRECAUTIONS**

1. Samples/reagent contains Human origin material, *HANDLE AS IF INFECTIOUS*! Universal precautions should be observed.
2. Reagent containing ProClin (for HBsAg ) may cause irritation. Avoid contact with skin. Rinse immediately with plenty soap and water
3. Do not place solutions containing sodium hypochlorite in the autoclave.

**ASSAY PROCEDURE**

|  |  |
| --- | --- |
| METHOD  | OPERATIONS |
| Controls/sample | 100µl |
| Conjugate addition (R6+R7) | 50µl |
| 1ST incubation  | 1hr 30 minutes |
| Temperature | 37oc $\pm $2  |
| Wash step | 5 cycles |
| Development solution(R8+R9) | 100µl |
| 2ND incubation (Dark) | 30 minutes |
| Temperature  | 18-30oc |
| Stop solution | 100$µ$l |
| 3rd incubation (work bench) | 4 minutes |
| Temperature | 18-30oc |
| Reading OD | 450nm |

**CALCULATION/RESULT INTERPRETATION**

Findthe mean value of the negative controls O.D.

 (NCI)OD + (NC2)OD + (NC3)OD

 3 = ODR3

Calculation of the cut-off value (CO)

* For each Method, the cut-off value is equal to: OD R3+0.050

Calculation of ratio sample

For each sample, calculate the ratio:

* Ratio S/CO = OD samples

 Cut-off value

**Interpretation of the results**

* Samples with ratio values lower than 1 are considered to be negative by the Monolisa™ HBs Ag ULTRA.
* Samples with ratio values equal to or greater than 1 are considered to be initially positive by the Monolisa HBs Ag ULTRA. They should be retested in duplicate before final interpretation.

**LIMITATION**

* Poor washing may provide wrong results with OD above 0.100 in the absence of development solution
* In very rare cases of genetic mutations a negative sample could have detectable viral DNA.

**This SOP has been read and understood by:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name**  | **Date**  | **Name** | **Date**  |
|  |  | 8. |  |
|  |  | 9. |  |
|  |  | 10. |  |
|  |  | 11. |  |
|  |  | 12. |  |
|  |  | 13. |  |
|  |  | 14. |  |