5.0 PRECAUTIONS

For In Vitro Diagnostic Use

Not for External or Internal Use in Animals

All products that contain human serum have also been shown to contain Hepatitis B Surface Antigens. HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that intradermal agents are absent, all human serum products should be handled as potentially infectious and capable of transmitting diseases. Crush samples for handling Biohazard products can be found in the FCBCDS Precaution statements.

5.1 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or plasma in tubes, and the usual precautions in the collection of these specimens shall be observed. For analytical purposes, specimens for antibody determination shall be collected in plain red-top tubes without anticoagulants or containing sodium citrate or EDTA. For the determination of antigen, a specimen should be collected in a yellow-top tube containing 3.8% sodium citrate. The tubes shall be left to clot for 1 hour specimens. Centrifuge the specimen to separate the serum or plasma from the clot.

In patients receiving therapy with high biological doses (i.e. ‘Single’), no sample should be taken for up to 7 days after the last biotherapy administration, preferably overnight to ensure fasting sampling.

Samples may be refrigerated at 2-8°C for a maximum period of 5 days (if the specimen was collected in citrate, samples may be stored at room temperature for 30 days). Each sample should be stored at a temperature of 2-8°C until the serum reference calibrator, control or the patient sample is added to the reagents and then incubated at room temperature for 15 minutes. Mix well.

9.0 QUALITY CONTROL

Each laboratory should ensure that critical levels are set for each assay preparation. Times controls should be treated an unknown and values determined in each test procedure performed. Quality control should be monitored to ensure that the performance of the requested samples. Statistical methods should be used to ascertain trends. Significant deviation from established performance can indicate uncontrolled variation in experimental conditions or degradation of reagents. Fresh reagents should be used to determine the reason for variations.

6.0 REAGENT PREPARATION

6.1 Materials

- Glassware and plasticware free from metal and plasticizers.
- Microplate, Colorimetric.
- Reagent Grade Water, and Buffer.
- Microplate reader (450nm absorbance capability).
- Microplate strips or microtiter plates.
- Pipette(s) capable of delivering 0.025, 0.050 & 0.100ml (25, 50 & 100µl) volumes with accuracy.
- 72°C incubator.
- 8°C refrigeration.

6.2 Reagents

- 0.05 M Tris-HCl, pH 7.4 (buffer). Prepare fresh on the day of use.
- 0.0025 M Coomassie Brilliant Blue R250 in 23% (v/v) methanol.
- 0.005 M sodium dodecyl sulfate (SDS).
- Microplate reader with 450nm and 620nm wavelength absorbance capability.
- 96-well microplates coated with streptavidin and packaged in an aluminum bag.

6.3 Procedure

1. Select the number of coated wells needed by formatting the microplate for each assay and adding corresponding serum reference calibrators or controls.
2. Pipette 0.025µl (25µl) of the appropriate serum reference standard or control.
3. Add 0.0100µl (10µl) of the appropriate sample to each well. It is very important to use the correct 'Sample Reagent' for each assay for accurate results.
4. Swell the microwells for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Draw the best-fit curve through the plotted points.
7. Measure the absorbance (mA) of calibrator 'F' should be > 1.3
8. Measure the absorbance (mA) of calibrator 'E' should be > 1.2
9. Measure the absorbance (mA) of calibrator 'D' should be > 1.1
10. Measure the absorbance (mA) of calibrator 'C' should be > 1.0
11. Measure the absorbance (mA) of calibrator 'B' should be > 0.9
12. Measure the absorbance (mA) of calibrator 'A' should be > 0.8
13. The absorbance (mA) of calibrator 'F' should be > 1.3
14. The absorbance (mA) of calibrator 'E' should be > 1.2
15. The absorbance (mA) of calibrator 'D' should be > 1.1
16. The absorbance (mA) of calibrator 'C' should be > 1.0
17. The absorbance (mA) of calibrator 'B' should be > 0.9
18. The absorbance (mA) of calibrator 'A' should be > 0.8
19. The absorbance (mA) of calibrator 'F' should be > 1.3
20. The absorbance (mA) of calibrator 'E' should be > 1.2
21. The absorbance (mA) of calibrator 'D' should be > 1.1
22. The absorbance (mA) of calibrator 'C' should be > 1.0
23. The absorbance (mA) of calibrator 'B' should be > 0.9
24. The absorbance (mA) of calibrator 'A' should be > 0.8
25. Absorbance (mA) should be > 0.8

6.4 Data Analysis

1. The absorbance (OD) of calibrator 'F' should be > 1.3
2. The absorbance (OD) of calibrator 'E' should be > 1.2
3. The absorbance (OD) of calibrator 'D' should be > 1.1
4. The absorbance (OD) of calibrator 'C' should be > 1.0
5. The absorbance (OD) of calibrator 'B' should be > 0.9
6. The absorbance (OD) of calibrator 'A' should be > 0.8

7.0 TEST PROCEDURE

7.1 General Procedure

- Start by filling the wells with 200µl of the appropriate serum reference standard.
- Mix well, and cover. Attach the microplate to the microplate reader and perform the incubation at room temperature (25°C) for 15 minutes. Mix well.

7.2 Pipetting

- Pipette 0.025ml (25µl) of the appropriate serum reference standard or control.
- 0.0100µl (10µl) of the appropriate sample.
- Add the diluent (330µl; 290µl of diluent and 40µl of 0.05% Tween 20) to the sample in each of the microtiter wells, mix well.

7.3 Measurement

- Measure the absorbance (mA) of calibrator 'F' should be > 1.3
- Measure the absorbance (mA) of calibrator 'E' should be > 1.2
- Measure the absorbance (mA) of calibrator 'D' should be > 1.1
- Measure the absorbance (mA) of calibrator 'C' should be > 1.0
- Measure the absorbance (mA) of calibrator 'B' should be > 0.9
- Measure the absorbance (mA) of calibrator 'A' should be > 0.8

7.4 Sample Calculations

- The absorbance (mA) of calibrator 'F' should be > 1.3
- The absorbance (mA) of calibrator 'E' should be > 1.2
- The absorbance (mA) of calibrator 'D' should be > 1.1
- The absorbance (mA) of calibrator 'C' should be > 1.0
- The absorbance (mA) of calibrator 'B' should be > 0.9
- The absorbance (mA) of calibrator 'A' should be > 0.8

7.5 Calculation of Results

- Measure the absorbance (mA) of calibrator 'F' should be > 1.3
- Measure the absorbance (mA) of calibrator 'E' should be > 1.2
- Measure the absorbance (mA) of calibrator 'D' should be > 1.1
- Measure the absorbance (mA) of calibrator 'C' should be > 1.0
- Measure the absorbance (mA) of calibrator 'B' should be > 0.9
- Measure the absorbance (mA) of calibrator 'A' should be > 0.8

8.0 SPECIMEN COLLECTION AND PREPARATION

8.1 Specimen Collection

- The specimen shall be blood, serum, or plasma in tubes, and the usual precautions in the collection of these specimens shall be observed. For analytical purposes, specimens for antibody determination shall be collected in plain red-top tubes without anticoagulants or containing sodium citrate or EDTA. For the determination of antigen, a specimen should be collected in a yellow-top tube containing 3.8% sodium citrate. The tubes shall be left to clot for 1 hour specimens. Centrifuge the specimen to separate the serum or plasma from the clot.

8.2 Specimen Precautions

- Specimen shall be handled as potentially infectious and capable of transmitting diseases. Crush samples for handling Biohazard products can be found in the FCBCDS Precaution statements.

8.3 Specimen Storage

- The specimen shall be handled as potentially infectious and capable of transmitting diseases. Crush samples for handling Biohazard products can be found in the FCBCDS Precaution statements.

8.4 Specimen Stability

- The specimen shall be handled as potentially infectious and capable of transmitting diseases. Crush samples for handling Biohazard products can be found in the FCBCDS Precaution statements.

8.5 Specimen Assay

- The specimen shall be handled as potentially infectious and capable of transmitting diseases. Crush samples for handling Biohazard products can be found in the FCBCDS Precaution statements.

8.6 Specimen Assay

- The specimen shall be handled as potentially infectious and capable of transmitting diseases. Crush samples for handling Biohazard products can be found in the FCBCDS Precaution statements.

8.7 Specimen Assay

- The specimen shall be handled as potentially infectious and capable of transmitting diseases. Crush samples for handling Biohazard products can be found in the FCBCDS Precaution statements.

8.8 Specimen Assay

- The specimen shall be handled as potentially infectious and capable of transmitting diseases. Crush samples for handling Biohazard products can be found in the FCBCDS Precaution statements.
The least square regression equation and correlation coefficient indicates excellent system and the reference methods are indicated by the closeness of the mean values. The mean value, standard deviation and coefficient of variation for each of these control sera system were determined by analyses on three different levels of pooled sera. The number, the within and between assay precision of Cancer Panel method with a population indigenous to the area in which the laboratory is located.

A study of an apparent normal adult population was undertaken to determine expected concentrations. Clinical and non-clinical specimens were assayed. The total number of such concentrations.

It is important to keep in mind that establishment of a range of values, which can be used to define 'normal', is not a 'closed' process. Independently of a multiplicity of factors the specificity of the method, the population assayed and the level of the control sera etc. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only on one level or range for determining the method with a population indigenous to the area in which the laboratory is located.

**14.1 Precision**

The within and between precision of Cancer Panel VAST® AccuBind® ELISA test system were determined by analyzing on three different levels of pooled sera. The number, the within and between assay precision of Cancer Panel method with a population indigenous to the area in which the laboratory is located.

**14.2 Sensitivity**

The sensitivity of the VAST® AccuBind® ELISA test system has sensitivity for different analyses as listed in the following Table 11. The sensitivity was assayed by determining the variation of the serum concentrations. The sensitivity is determined by calculating the minimum dose.

**14.3 Accuracy**

The Cancer Panel VAST® AccuBind® ELISA test system was compared with reference methods. Clinical and non-clinical specimens were assayed. The total number of each specimen was 490. The least square regression equation and the correlation coefficient were computed for AFP, CEA and PSA assays in comparison with the reference method. The data obtained is shown in Table 6 - 10.

**14.5 Linearity & Assay Effect**

Three different lots of reagent preparations of the Cancer Panel VAST® AccuBind® ELISA test system were used to assess the linearity and linearity effect of the test. The test showed a good dose recovery of 97% to 104% when linear dilutions of very high concentrations, in pooled sera were assayed with Cancer Panel VAST® AccuBind® ELISA test system.

Massive concentrations were used for spiking in pooled human sera samples. Cancer Panel VAST® AccuBind® ELISA test system did not show any high dose hook effect with following concentrations of respective analytes.

**REFERENCES**


