HDV Ab

Competitive Enzyme Immunoassay for the determination of antibodies to Hepatitis Delta Virus in human serum and plasma

- for “in vitro” diagnostic use only -

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HDV Ab

A. INTENDED USE
Competitive Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis Delta Virus or HDV in human plasma and sera with a "two-steps" methodology. The kit is used for the follow-up of patients infected by HDV. For "in vitro" diagnostic use only.

B. INTRODUCTION
The Hepatitis Delta Virus or HDV is a RNA defective virus composed of a core presenting the delta-specific antigen, encapsulated by HBsAg, that requires the helper function of HBV to support its replication. Infection by HDV occurs in the presence of acute or chronic HBV infection. When acute delta and acute HBV simultaneously occur, the illness becomes severe and clinical and biochemical features may be indistinguishable from those of HBV infection alone. In contrast, a patient with chronic HBV infection can support HDV replication indefinitely, usually with a less severe illness appearing as a clinical exacerbation. The determination of HDV specific serological markers (HDV Ag, HDV Ab, HDV IgM and HDV IgG) represents in these cases an important tool to the clinician for the classification of the clinical exacterberation, for the follow up of infected patients and their treatment. The detection of HDV total antibodies allows the classification of the illness and the monitoring of the seroconversion event.

C. PRINCIPLE OF THE TEST
Anti-HDV antibodies, if present in the sample, compete with a virus-specific polyclonal IgG, labeled with peroxidase (HRP), for a fixed amount of rec-HDV coated on the microplate. The test is carried out with a two steps incubation competitive system. First the sample is added to the plate and specific anti HDV antibodies bind to the adsorbed antigen. After washing, an enzyme conjugated polyclonal antibody to HDV is added and binds to the free portion of the antigen coated. After washing a chromogen/substrate mixture is dispensed. The concentration of the bound enzyme on the solid phase becomes inversely proportional to the amount of anti-HDV antibodies in the sample and its activity is detected by the added chromogen/substrate. The concentration of HDV-specific antibodies in the sample is determined by means of a cut-off value that allows for the semi quantitative detection of anti-HDV antibodies.

D. COMPONENTS
Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: 8x12 microwell strips coated with recombinant HDV-specific antigen and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Negative Control: Ready to use. Contains goat serum proteins, 100 mM Tris-HCl buffer pH 7.4 +/-0.1, 0.09% Sodium Azide and 0.1% Kathon GC as preservatives. The negative control is colour coded pale yellow.

3. Positive Control: Ready to use. Contains goat serum proteins, high titer anti HDV antibodies, 100 mM Tris-HCl buffer pH 7.4 +/-0.1, 0.09% Sodium Azide and 0.1% Kathon GC as preservatives. The positive control is colour coded green.

4. Calibrator: n°1 vial. Lyophilised. To be dissolved with EIA grade water as reported in the label. Contains bovine serum proteins, low titer human antibodies to HDV, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives. Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

5. Wash buffer concentrate: 1x60ml/bottle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0 +/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

6. Enzyme conjugate: 1x16ml/vial. Ready-to-use solution. Contains 5% bovine serum albumine, 10 mM tris buffer pH 6.8 +/-0.1, Horseradish peroxidase conjugated polyclonal antibody to HDV in presence of 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives. The component is colour coded red.

7. Chromogen/Substrate: 1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% DMSO, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide of H2O2. Note: To be stored protected from light as sensitive to strong illumination.

8. Sulphuric Acid: 1x15ml/vial. Contains 0.3 M H2SO4 solution. Attention: Irritant (Xi R36/38; S2/26/30)

Plate sealers n°2

Instructions for Use n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropettites in the range 10-1000 ul and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and if possible with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health’s publication: “Biosafety in Microbiological and Biomedical Laboratories”, ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminant such as dust or air-born microbial
agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (TMB/H2O2) from strong light and avoid vibration of the bench surface where the test is undertaken.

5. Upon receipt, store the kit at +2.8°C into a temperature controlled refrigerator or cold room.

6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.

7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.

8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.

9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.

10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels.

11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health’s publication: “Biosafety in Microbiological and Biomedical Laboratories”, ed. 1984.

12. The use of disposable plastic labware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.

13. Waste produced during the use of the kit has to be discarded as they could generate false results. Microbiological and Biomedical Laboratories, Atlanta, U.S in compliance with what reported in the Institutes of Health’s publication: “Biosafety in Microbiological and Biomedical Laboratories”, ed. 1984.

G. SPECIMEN: PREPARATION AND RECOMMENDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.

2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate.

3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.

4. Haemolysed (red) and visibly hyperlipemic ("milk y") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.

5. Sera and plasma can be stored at +2.8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.

6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8μ filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

1. Antigen coated microwells:

   Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing. In this case, call Dia.Pro’s customer service. Unused strips have to be placed back into the aluminium pouch, with the desiccant supplied, firmly zipped and stored at +2-8°C. When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:

   Ready to use. Mix well on vortex before use.

3. Positive Control:

   Ready to use. Mix well on vortex before use.

4. Calibrator:

   Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.

   Note: The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C.

5. Wash buffer concentrate:

   The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200ml and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

   Note: Once diluted, the wash solution is stable for 1 week at +2-8°C.

6. Enzyme conjugate:

   Ready to use. Mix well on vortex before use. Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

7. Chromogen/Substrate:

   Ready to use. Mix well on vortex before use. Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces. If this component has to be transferred use only plastic, and if possible, sterile disposable container

8. Sulphuric Acid:

   Ready to use. Mix well on vortex before use.

   Attention: Irritant (Xi R36/38; S2/26/30)

   Legenda: R 36/38 = Irritating to eyes and skin.
   S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of ±2%.

2. The ELISA incubator has to be set at +37°C (tolerance of ±0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.

3. The ELISA washer is extremely important to the overall performances of the assay. The washer has to be carefully validated and correctly optimized using the kit controls/calibrator and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls/calibrator and well characterized negative and positive reference samples, and check to match the values reported below in the sections "Validation of Test" and "Assay Performances". Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.

4. Incubation times have a tolerance of ±5%.

5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and ideally with a second filter (620-630nm) for blanking purposes. Its standard performances should be (a) bandwidth < 10 nm; (b) absorbance range from 0 to > 2.0; (c) linearity to > 2.0; repeatability > 1%.

6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions.

7. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

1. Place the required number of strips in the microplate holder. Leave A1 well empty for the operation of blanking. Store the other strips into the bag in presence of the desiccant at +2.8°C, sealed.

2. Pipette 100 µl of Negative Control in triplicate, 100 µl of Calibrator in duplicate, 100 µl of Positive Control in single and then 100µl of samples. Check that controls/calibrator and samples have been correctly added. Then incubate the microplate at +37°C for 60 min.

3. Wash the microplate as reported in section I.3.

4. In all the wells except A1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Then incubate the microplate at +37°C for 60 min.

**Important note:** Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.

5. Wash the microplate as described.

6. Pipette 100 µl TMB/H₂O₂ mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at room temperature for 20 min.

**Important note:** Do not expose to strong direct light as a high background might be generated.

7. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step n° 6 to stop the enzymatic reaction. Addition of the stop solution will turn the negative control and negative samples from blue to yellow.

8. Measure the colour intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and if possible a 620-630nm filter (background subtraction), blanking the instrument on A1.
Important notes:
1. If the second filter is not available, ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.
2. Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.

N. ASSAY SCHEME

<table>
<thead>
<tr>
<th>Controls/Calibrator</th>
<th>100 ul</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>100 ul</td>
</tr>
<tr>
<td>1st incubation</td>
<td>60 min</td>
</tr>
<tr>
<td>Temperature</td>
<td>+37°C</td>
</tr>
<tr>
<td>Washing step</td>
<td>4-5 cycles</td>
</tr>
<tr>
<td>Enzyme Conjugate</td>
<td>100 ul</td>
</tr>
<tr>
<td>2nd incubation</td>
<td>60 min</td>
</tr>
<tr>
<td>Temperature</td>
<td>+37°C</td>
</tr>
<tr>
<td>Washing step</td>
<td>4-5 cycles</td>
</tr>
<tr>
<td>TMB/H2O2 mix</td>
<td>100 ul</td>
</tr>
<tr>
<td>3rd incubation</td>
<td>20 min</td>
</tr>
<tr>
<td>Temperature</td>
<td>r.t.</td>
</tr>
<tr>
<td>Sulphuric Acid</td>
<td>100 ul</td>
</tr>
<tr>
<td>Reading OD</td>
<td>450nm &amp; 620nm</td>
</tr>
</tbody>
</table>

An example of dispensation scheme is reported in the table below:

<table>
<thead>
<tr>
<th>Microplate</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>BLK S2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>NC S3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>NC S4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>NC S5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>CAL S6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>CAL S7</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>G</td>
<td>PC S8</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>S1 S9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legenda: BLK = Blank       NC = Negative Control
CAL = Calibrator          PC = Positive Control
S = Sample

O. INTERNAL QUALITY CONTROL
A check is performed on the controls/calibrator any time the kit is used in order to verify whether the expected OD450nm or S/Co values have been matched in the analysis.

Ensure that the following parameters are met:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank well (0100 OD450nm value)</td>
<td>&lt; 0.100 OD450nm value</td>
</tr>
<tr>
<td>Negative Control (NC)</td>
<td>&gt; 1.000 OD450nm after blanking</td>
</tr>
<tr>
<td>Enzyme Conjugate</td>
<td>If lower carefully control the washing procedure and decrease the number of cycles or the soaking time coefficient of variation &lt; 30%</td>
</tr>
<tr>
<td>Calibrator</td>
<td>NC/10 &lt; OD450nm &lt; NC/5</td>
</tr>
<tr>
<td>Positive Control</td>
<td>OD450 nm &lt; NC/10</td>
</tr>
</tbody>
</table>

If the results of the test match the requirements stated above, proceed to the next section.
If they don’t, do not proceed any further and perform the following checks:

Q. INTERPRETATION OF RESULTS
Results are interpreted as ratio between the cut-off value and the sample OD450nm or Co/S. Results are interpreted according to the following table:

<table>
<thead>
<tr>
<th>Co/S</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.9</td>
<td>Negative</td>
</tr>
<tr>
<td>0.9 – 1.1</td>
<td>Equivocal</td>
</tr>
<tr>
<td>&gt; 1.1</td>
<td>Positive</td>
</tr>
</tbody>
</table>

A negative result indicates that the patient has not been infected by HDV.
Any patient showing an equivocal result should be re-tested on a second sample taken 1-2 weeks after the initial sample.
A positive result is indicative of HDV infection and therefore the patient should be treated accordingly.

**Important notes:**
1. Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.
2. When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.
3. Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.

An example of calculation is reported below.

The following data must not be used instead or real figures obtained by the user.

- **Negative Control:** 2.100 – 2.200 – 2.000 OD450nm
- **Mean Value:** 2.100 OD450nm
- **Higher than 1.000 – Accepted**

- **Positive Control:** 0.100 OD450nm
- **Lower than NC/10 – Accepted**

**Cut-Off = (2.100 + 0.100) / 5 = 0.440**

### R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

#### 1. LIMIT OF DETECTION:

In absence of an international standard, the sensitivity of the assay has been calculated by means of the product named Accurun n° 127 supplied by Boston Biomedica Inc. – USA.

The table below reports the OD450nm shown by three replicates in three separate runs for three lots of product, is used to determine the specificity. No false reactivity due to the preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.

Results are briefly reported in the tables below:

### 2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

The diagnostic performances were evaluated in a clinical trial conducted by the Department of Gastro-Hepatology, Prof. M.Rizzetto, S.Giovanni Battista hospital, Torino, Italy, on more than 400 samples against a reference kit. Negative, positive and potentially interfering samples were examined in the trial.

Both plasma, derived with different standards of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.

### 3. PRECISION

The mean values obtained from a study conducted on two samples of different anti-HDV antibody reactivity, examined in 16 replicates in three separate runs for three lots of product, is reported below:

- **DAB.CE: lot #1102**
- **DAB.CE: lot #0103**
- **DAB.CE: lot #0403**

### Co/S values

<table>
<thead>
<tr>
<th>Accurun #127</th>
<th>OD450nm</th>
<th>Co/S value</th>
<th>OD450nm</th>
<th>Co/S value</th>
<th>OD450nm</th>
<th>Co/S value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x</td>
<td>0.171</td>
<td>3.0</td>
<td>0.163</td>
<td>2.9</td>
<td>0.156</td>
<td>2.8</td>
</tr>
<tr>
<td>2x</td>
<td>0.187</td>
<td>2.7</td>
<td>0.176</td>
<td>2.6</td>
<td>0.179</td>
<td>2.5</td>
</tr>
<tr>
<td>4x</td>
<td>0.230</td>
<td>2.3</td>
<td>0.220</td>
<td>2.1</td>
<td>0.202</td>
<td>2.2</td>
</tr>
<tr>
<td>8x</td>
<td>0.298</td>
<td>1.7</td>
<td>0.285</td>
<td>1.6</td>
<td>0.271</td>
<td>1.6</td>
</tr>
<tr>
<td>16x</td>
<td>0.417</td>
<td>1.2</td>
<td>0.405</td>
<td>1.1</td>
<td>0.402</td>
<td>1.1</td>
</tr>
<tr>
<td>32x</td>
<td>0.514</td>
<td>1.0</td>
<td>0.490</td>
<td>0.9</td>
<td>0.482</td>
<td>0.9</td>
</tr>
<tr>
<td>64x</td>
<td>0.717</td>
<td>0.7</td>
<td>0.700</td>
<td>0.7</td>
<td>0.705</td>
<td>0.6</td>
</tr>
<tr>
<td>128x</td>
<td>1.063</td>
<td>0.5</td>
<td>1.006</td>
<td>0.5</td>
<td>1.015</td>
<td>0.4</td>
</tr>
<tr>
<td>CTRL (-)</td>
<td>2.484</td>
<td></td>
<td>2.261</td>
<td></td>
<td>2.114</td>
<td></td>
</tr>
</tbody>
</table>

### 4. LIMIT OF DETECTION:

In absence of an international standard, the sensitivity of the assay has been calculated by means of the product named Accurun n° 127 supplied by Boston Biomedica Inc. – USA.

The table below reports the OD450nm shown by three replicates in three separate runs for three lots of product, is used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.

Results are briefly reported in the tables below:

### Sensitivity > 98 %

### Specificity > 98 %
The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte. This test is suitable only for testing single samples and not pooled ones. Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient’s clinical history, symptomatology, as well as other diagnostic data should be considered.

REFERENCES