PLATELIA™ DENGUE NS1 Ag

1 plate - 96

QUALITATIVE OR SEMI-QUANTITATIVE DETECTION OF DENGUE VIRUS NS1 ANTIGEN IN HUMAN SERUM OR PLASMA BY ENZYME IMMUNOASSAY

CE

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1- CLINICAL VALUE

Dengue is an endemic disease affecting tropical and subtropical regions around the world. It is considered as the most important arboviral disease in terms of morbidity, mortality and socio-economical costs. Global prevalence of dengue has increased dramatically in recent decades and the disease is now endemic in more than 100 countries, and potentially concern 40% of earth population. The World Health Organization estimates that there are about 50 to 100 million cases of dengue infections worldwide every year, which results in 250,000 to 500,000 severe complicated forms of the disease and 24,000 deaths each year.

Dengue virus is transmitted by mosquito, mainly Aedes aegypti and Aedes albopictus. There are four distinct serotypes (DEN-1, DEN-2, DEN-3, DEN-4). Primary infection induces a life-long protective immunity to the homologous serotype, but confers only partial and transient protection against the other three serotypes in case of re-infection (secondary infection).

Infection with dengue virus causes a broad spectrum of illnesses, ranging from asymptomatic infection, undifferentiated fever and classical dengue fever (DF), to the more severe forms, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) with high rates of morbidity and mortality. DF is characterised by fever lasting 3-5 days, headache, muscle and joint pain, rash, but usually patient recovery. DHF or DSS, which mainly occur in patient previously infected with the virus, present similar symptoms to DF, but are followed by increased vascular permeability and hemorrhagic signs leading to reduce blood pressure, hypovolemia, vascular collapsus and death.

The most challenging problem associated with infected patient management is rapid and specific detection of dengue virus during acute phase in order to implement timely clinical treatment. Isolation and identification of the virus or detection of viral nucleic acid allow early diagnostic during febrile phase, but both methods need a specialized laboratory and results are not immediate. Detection of dengue virus-specific antibodies are commonly used for routine diagnostic. However, antibodies appear after symptoms onset. In primary infection, IgM and IgG arise approximatively 5 and 14 days respectively after symptom onset.
In secondary infection, IgM levels are low or undetectable while IgG rise 1-2 days after symptom onset with higher levels than in primary infection. More recently, detection in patients sera of circulating dengue virus nonstructural protein NS1 has been described as an alternative method for early diagnosis. NS1 antigen was found circulating from the first day and up to 9 days after the onset of fever, with comparable levels observed in primary and secondary infections.

2- **PRINCIPLE**

Platelia™ Dengue NS1 Ag is a one step sandwich format microplate enzyme immunoassay for the qualitative or semi-quantitative detection of Dengue virus NS1 antigen in human serum or plasma. The test uses murine monoclonal antibodies (MAb) for capture and revelation. Samples and controls are directly and simultaneously incubated with the conjugate for 90 minutes at 37°C within the microplate wells sensitised with MAb. If NS1 antigen is present in the sample, an immune-complex MAb - NS1 - MAb/peroxidase will be formed. After a washing step, the presence of immune-complex is demonstrated by distribution in each well of a chromogenic solution initiating a color development reaction. After 30 minutes of incubation at room temperature, the enzymatic reaction is stopped by addition of an acid solution. The optical density reading obtained with a spectrophotometer set at 450/620 nm is proportional to the amount of NS1 antigen present in the sample. The presence of NS1 antigen in an individual sample is determined by comparing the optical density reading of the sample to the optical density of the calibrator.
### 3- PRODUCT INFORMATION

<table>
<thead>
<tr>
<th>Label</th>
<th>Nature of reagents</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R1</strong> Microplate</td>
<td>Microplate (Ready-to-use): 12 strips with 8 wells each, coated with anti-NS1 MAb, in vacuum sealed bag</td>
<td>1</td>
</tr>
<tr>
<td><strong>R2</strong> Concentrated Washing Solution (20X)</td>
<td>Concentrated Washing Solution (20X): TRIS-NaCl buffer (pH 7.4), Preservative: 0.04% ProClin™ 300</td>
<td>1 x 70 ml</td>
</tr>
<tr>
<td><strong>R3</strong> Negative Control</td>
<td>Negative Control: Human serum negative for Dengue NS1 antigen. Preservative: 0.15% ProClin™ 300</td>
<td>1 x 1.0 ml</td>
</tr>
<tr>
<td><strong>R4</strong> Calibrator</td>
<td>Calibrator: TRIS-NaCl buffer (pH 8.0), Dengue NS1 antigen, bovine serum albumin, glycérol, E102, E122. Preservative: 0.15% ProClin™ 300</td>
<td>1 x 1.5 ml</td>
</tr>
<tr>
<td><strong>R5</strong> Positive Control</td>
<td>Positive Control: TRIS-NaCl buffer (pH 8.0), Dengue NS1 antigen, bovine serum albumin, glycérol, E102, E122. Preservative: 0.15% ProClin™ 300</td>
<td>1 x 1.0 ml</td>
</tr>
<tr>
<td><strong>R6</strong> Conjugate (50x)</td>
<td>Conjugate (50x): Anti-NS1 MAb coupled with horseradish peroxydase. Preservative: 0.15% ProClin™ 300</td>
<td>1 x 0.5 ml</td>
</tr>
<tr>
<td><strong>R7</strong> Diluent</td>
<td>Diluent (Ready-to-use): Phosphate buffer, Tween® 20, fetal calf serum. Preservative: 0.15% ProClin™ 300</td>
<td>1 x 22 ml</td>
</tr>
<tr>
<td><strong>R9</strong> Chromogen TMB</td>
<td>Chromogen (Ready-to-use): 3.3’5.5’ tetramethylbenzidine (&lt; 0.1%), H₂O₂ (&lt;1%)</td>
<td>1 x 28 ml</td>
</tr>
<tr>
<td><strong>R10</strong> Stopping Solution</td>
<td>Stopping Solution (Ready-to-use): 1N sulfuric acid solution</td>
<td>1 x 28 ml</td>
</tr>
</tbody>
</table>

For storage conditions and expiration date, refer to the indications mentioned on the box.
4- WARNING AND PRECAUTIONS

The reliability of the results depends on correct implementation of the following Good Laboratory Practices:

• Do not use expired reagents.
• Do not mix reagents from different lots within a given test run.

REMARK: For Washing Solution (R2, label identification: 20x colored green), Chromogen (R9, label identification: TMB colored turquoise) and Stopping Solution (R10, label identification: 1N colored red), it is possible to use other lots than those contained in the kit, provided these reagents are strictly equivalent and the same lot is used within a given test run.

REMARK: It is not permissible to use Diluent (R7) from lots other than provided in the kit.

REMARK: In addition, the Washing Solution (R2, label identification: 20x colored green) can be mixed with the 2 other washing solutions included in various Bio-Rad reagent kits (R2, label identifications: 10x colored blue or 10x colored orange) when properly reconstituted, provided only one mixture is used within a given test run.

• Before use, allow reagents to reach room temperature (+18-30°C).
• Carefully reconstitute or dilute the reagents avoiding any contamination.
• Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugate.
• Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
• Do not allow the microplate to dry between the end of the washings operation and the reagent distribution.
• The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or substrate solutions.
• Chromogen Solution (R9) should be colorless. The appearance of a blue color indicates that the reagent cannot be used and must be replaced.
• Use a new pipette tip for each sample.
• Washing the microplate is a critical step in the procedure: follow the recommended number of washings cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washings may lead to inaccurate results.
• Never use the same container to distribute conjugate and development solution.
• Check the pipettes and other equipment for accuracy and correct operations.
• Do not change the assay procedure.

HEALTH AND SAFETY INSTRUCTIONS
• Human origin material used in the preparation of the reagents has been tested and found non-reactive for hepatitis B surface antigen (HBs Ag), antibodies for hepatitis C virus (anti-HCV) and to human immunodeficiency virus (anti-HIV1 and anti-HIV2). Because no method can absolutely guarantee the absence of infectious agents, handle reagents of human origin and patient samples as potentially capable of transmitting infectious diseases.

• Any material, including washings solution, that comes directly in contact with samples and reagents containing materials of human origin, should be considered capable of transmitting infectious diseases.
• Wear disposable gloves when handling reagents.
• Do not pipette by mouth.
• Avoid spilling samples or solutions containing samples. Spills must be rinsed with bleach diluted to 10%. In the event of a spill with an acid, it must be first neutralized with sodium bicarbonate, then cleaned with bleach diluted at 10% and dried with absorbent paper. The material used for cleaning must be discarded in a contaminated residue container.
• Samples of human origin, as well as contaminated material and products should be discarded after decontamination, either by immersion in bleach at the final concentration of 5% of sodium hypochloride for 30 minutes, or by autoclaving at 121°C for 2 hours minimum. Autoclaving for at least one hour at 121°C is the best method to inactivate the HIV viruses and the HB viruses.

CAUTION: Do not introduce solutions containing sodium hypochloride into the autoclave
• Avoid any contact of the substrate buffer, chromogen and stopping solution with skin and mucosa (risk of toxicity, irritation or burn).
• Chemicals should be handled and disposed of in accordance with Good Laboratory Pratices.
• For hazard and precaution recommendations related to some chemical components in this test kit, please refer to the pictogram(s) mentioned on the labels and the information supplied at the end of instruction for use. The Safety Data Sheet is available on www.bio-rad.com.
5- SPECIMEN COLLECTION, PREPARATION AND STORAGE

1. Serum and plasma (EDTA, citrate, heparin) are the recommended sample types.

2. Observe the following recommendations for handling, processing and storing blood samples:
   • Collect all blood samples observing routine precaution for venipuncture.
   • For serum, allow samples to clot completely before centrifugation.
   • Keep tubes stoppered at all times.
   • After centrifugation, separate the serum or plasma from the clot or red cells in a tightly stoppered storage tube.
   • The specimen can be stored at +2-8°C if test is performed within 24 hours.
   • If the test will not be completed within 24 hours, or for shipment of samples, freeze at –20°C or colder.
   • Do not use samples that have been thawed more than three times. Previously frozen specimens should be thoroughly mixed after thawing prior to testing.

3. Samples containing 100 mg/L bilirubin and lipemic samples containing the equivalent of 36 g/L triolein (triglyceride) do not affect the results. Presence of albumin at 90g/L or hemolysed samples containing 10mg/mL of hemoglobin can potentially increase ratio of negative samples.

4. Do not heat the samples.

6- ASSAY PROCEDURE

6.1. MATERIALS REQUIRED BUT NOT PROVIDED
   • Vortex mixer.
   • Microplate reader equipped with 450 nm and 620 nm filters (*).
   • Water bath or equivalent microplate incubator thermostatically set at 37±1°C (*).
   • Manual, semi-automatic or automatic microplate washer (*).
   • Container for biohazard waste.
   • Sodium hypochloride (bleach) and sodium bicarbonate.
   • Sterile distilled or deionized water.
   • Graduated cylinders of 25 mL, 50 mL, 100 mL and 1000 mL capacity.
   • Disposable latex gloves.
   • Goggles or safety glasses.
   • Absorbent paper.
   • Automatic or semi-automatic, adjustable or preset, pipettes or mult pipettes to measure 50 µL, 100 µL, 300 µL and 1000 µL.
• Disposable tubes.
(*) Consult our technical department for detailed information about the recommended equipment.

6.2. REAGENTS RECONSTITUTION

• R1: Bring at room temperature (+18-30°C) before opening the bag. Return unused strips in the bag immediately and check the presence of desiccant. Carefully re-seal the bag and store it at +2-8°C.
• R2: Dilute 1/20 the Washing Solution R2 in distilled water: for example 50 mL of R2 and 950 mL of distilled water to get the ready-to-use Washing Solution. Prepare 350 mL of diluted Washing Solution for one plate of 12 strips if washing manually.
• R6+R7: Conjugate (R6) is concentrated 50x and must be homogenise before use. Dilute 1/50 with Diluent (R7). For one strip, dilute 20 µL of R6 qsp. 1.0 mL of R7. Multiply these volumes by 12 for one microplate.

6.3. STORAGE OF OPENED AND/OR RECONSTITUTED REAGENTS

The kit must be stored at +2-8°C. When the kit is stored at +2-8°C before opening, each component can be used until the expiration date indicated on the outer label of the kit.
• R1: Once opened, the strips remain stable for up to six weeks if stored at +2-8°C in the same carefully closed bag containing the desiccant.
• R2: Once diluted, the Washing Solution can be kept for 2 weeks at +2-30°C. Once opened, the concentrated Washing Solution stored at +2-30°C, in absence of contamination, is stable until the expiration date indicated on the label.
• R6+R7: Once diluted, the reconstituted solution is stable 8 hours at room temperature (+18-30°C).
• R3, R4, R5, R6, R7, R10: Once opened, reagents stored at +2-8°C, in absence of contamination, are stable until the expiration date indicated on the label.
• R9: Once opened and without any contamination, the reagent stored at +2-8°C is stable for up to 8 weeks.

6.4. PROCEDURE

Strictly follow the assay procedure.
Before use, allow reagents to reach room temperature (+18-30°C).
Use one negative control (R3), two calibrators (R4) and one positive control (R5) in each run to validate the assay results.
1. Carefully establish the distribution and identification plan for calibrator, controls and patient samples (S1, S2…) as indicated below:

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<tbody>
<tr>
<td>A</td>
<td>R3</td>
<td>S5</td>
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<td>B</td>
<td>R4</td>
<td>S6</td>
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<td>S1</td>
<td>S9</td>
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<td>F</td>
<td>S2</td>
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<tr>
<td>H</td>
<td>S4</td>
<td>S12</td>
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</tr>
</tbody>
</table>

2. Take the carrier tray and the strips (R1) out of the protective pouch (Refer to section 6.2).

3. Strictly following the indicated distribution sequence, distribute successively in the wells:
   - 50µL of diluent (R7)
   - 50µL of samples (calibrator, controls or patients)
   - 100µL of diluted conjugate (R6+R7)

   **N.B:** Diluent, sample and conjugate distributions can be visually controlled at this step of the manipulation. When adding the neat sample to the diluent, the color turns from yellow to orange. After adding the conjugate, the color turns from orange to green. This control could be altered when using diluted samples.

4. Cover the reaction microplate with an adhesive plate sealer, pressing firmly onto the plate to ensure a tight seal.

5. Incubate the microplate in a thermostat controlled water bath or microplate incubator for 90 ± 5 minutes at 37 ± 1°C.

6. Prepare the dilution of the washing solution (R2) (Refer to section 6.2).

7. At the end of the incubation period, remove the adhesive plate sealer.
   Aspirate the contents of all wells into a container for biohazard waste (containing sodium hypochloride). Wash microplate 6 times with washing solution (R2). Invert microplate and gently tap on absorbent paper to remove remaining liquid.

   **Note:** It is important to avoid reagent splashing during aspiration and washing steps.

8. Quickly distribute into each well and away from light 160 µL of Chromogen solution (R9). Allow the reaction to develop in the dark for 30 ± 5 minutes at room temperature (+18-30°C). Do not use adhesive plate sealer during this incubation.
9. Stop the enzymatic reaction by adding 100 µL of Stopping Solution (R10) in each well. Use the same sequence and rate of distribution as for the development solution.

10. Carefully wipe the plate bottom. Read the optical density at 450/620 nm using a plate reader within 30 minutes after stopping the reaction (The strips must always be kept away from light before reading).

11. Check all results for agreement between the reading and the distribution and identification of plate and samples.

7- CALCULATION AND INTERPRETATION OF RESULTS

7.1. CALCULATION OF THE CUT-OFF VALUE
The cut-off value CO corresponds to the mean value of the optical densities of the calibrator duplicates (R4).

7.2. CALCULATION OF THE SAMPLE RATIO
Sample result is expressed by Ratio using the following formula, where S is the optical density (OD) obtained on the sample:

• Sample Ratio = S/CO

7.3. QUALITY CONTROL
For validation of the assay, the following criteria must be met:

• Optical density values:
  - CO > 0,200

• Ratios:
  - R3 Ratio < 0,40 (R3 Ratio = OD_{R3} / CO)
  - R5 Ratio > 1,50 (R5 Ratio = OD_{R5} / CO)

If those specifications are not met, the test run should be repeated.

7.4. INTERPRETATION OF RESULTS
Refer to the table below for results interpretation.

<table>
<thead>
<tr>
<th>Sample Ratio</th>
<th>Result</th>
<th>Interpretation and recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio &lt; 0.50</td>
<td>Negative</td>
<td>The sample is considered non reactive for Dengue NS1 antigen.</td>
</tr>
<tr>
<td>0.50 ≤ Ratio &lt; 1.00</td>
<td>Equivocal</td>
<td>The sample is considered equivocal for Dengue NS1 antigen.</td>
</tr>
<tr>
<td>Ratio ≥ 1.00</td>
<td>Positive</td>
<td>The sample is considered reactive for Dengue NS1 antigen.</td>
</tr>
</tbody>
</table>

**Remark:** ODs obtained on highly reactive samples could reach the maximum OD readable on the spectrophotometer.
7.5. TROUBLE SHOOTING GUIDE
Non validated or non repeatable reactions are often caused by:
• Inadequate microplate washings.
• Contamination of negative samples by serum or plasma with a high concentration.
• Contamination of the development solution by oxidizing agents (bleach, metal ions...)
• Contamination of the stopping solution.

8- PERFORMANCES
8.1. SENSITIVITY – SPECIFICITY

• Sensitivity
Sensitivity was evaluated on 177 retrospective sera from patients with current dengue infection confirmed by RT-PCR. On this panel, the Platelia™ Dengue NS1 Ag assay was positive in 91% of cases (95% confidence interval: 85.8%-94.8%). By comparison, the sensitivity obtained with a commercialized Dengue IgM EIA assay was 17.5%.

Sensitivity was significantly higher in IgG negative samples from primary infection (sensitivity of 98.5%, n= 66) than in IgG positive samples (sensitivity of 85.6%, n=90) ($\chi^2$ test, p=0.004).

No significative difference was observed related to dengue serotypes as summarized in Table 1 below:

**Table 1:** Sensitivity of Platelia™ Dengue NS1 Ag related to virus serotype (n=177).

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number of sera</th>
<th>Sensitivity of Platelia™ Dengue NS1 Ag (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>93</td>
<td>88.9% (85.8% - 94.8%)</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>87.1% (70.1% - 96.3%)</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>100.0% (85.6% - 100.0%)</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>93.3% (77.9% - 97.9%)</td>
</tr>
</tbody>
</table>

The sensitivity of Platelia™ Dengue NS1 Ag was studied on sera from patients for which the onset of fever was documented. Highest sensitivities are obtained as soon as the clinical signs appear and stay high during febrile episodes as shown in Table 2.
Table 2: Sensitivity of Platelia™ Dengue NS1 Ag related to clinical signs apparition (n=177).

<table>
<thead>
<tr>
<th>Days after beginning of fever</th>
<th>Number of sera</th>
<th>Sensitivity of Platelia™ Dengue NS1 Ag</th>
<th>Sensitivity of Dengue IgM EIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>100.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>1</td>
<td>33</td>
<td>87.8%</td>
<td>5.1%</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>92.5%</td>
<td>6.1%</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>95.0%</td>
<td>15.0%</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>96.3%</td>
<td>48.1%</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>52.6%</td>
<td>94.1%</td>
</tr>
<tr>
<td>≥ 6</td>
<td>28</td>
<td>35.7%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

- **Specificity**

Specificity was evaluated on 618 specimens including samples from 563 blood donors and 55 hospitalized patients. No positive results were observed in the studied population, providing a specificity of 100.0% (95% confidence interval: 99.4% - 100.0%).

8.2. PRECISION

- **Intra-assay precision (repeatability)**

In order to evaluate intra-assay repeatability, one negative and three positive samples were tested 30 times during the same assay. The ratio (S/CO) was determined for each sample. Mean Ratio, Standard Deviation (SD), and Coefficient of Variation (%CV) for each of the four specimens are listed in Table 3 below.

Table 3: Intra-assay precision.

<table>
<thead>
<tr>
<th>N=30</th>
<th>Negative Sample</th>
<th>Weak Positive Sample</th>
<th>Medium Positive Sample</th>
<th>High Positive Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample Ratio (S/CO)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.10</td>
<td>1.32</td>
<td>3.79</td>
<td>6.24</td>
</tr>
<tr>
<td>SD</td>
<td>0.01</td>
<td>0.08</td>
<td>0.29</td>
<td>0.45</td>
</tr>
<tr>
<td>% CV</td>
<td>13.1</td>
<td>6.2</td>
<td>7.7</td>
<td>7.2</td>
</tr>
</tbody>
</table>
**Inter-assay precision (reproducibility)**

In order to evaluate inter-assay reproducibility, each of four specimens (one negative and three positive samples) was tested in duplicate, two runs a day, over a twenty day period. The ratio (S/CO) was determined for each sample. Mean Ratio, Standard Deviation (SD), and Coefficient of Variation (%CV) for each of the four specimens are listed in Table 4 below.

**Table 4: Inter-assay precision.**

<table>
<thead>
<tr>
<th>N=40</th>
<th>Negative Sample</th>
<th>Weak Positive Sample</th>
<th>Medium Positive Sample</th>
<th>High Positive Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample Ratio (S/CO)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.10</td>
<td>1.17</td>
<td>3.85</td>
<td>6.20</td>
</tr>
<tr>
<td>SD</td>
<td>0.03</td>
<td>0.21</td>
<td>0.67</td>
<td>0.96</td>
</tr>
<tr>
<td>% CV</td>
<td>33.8</td>
<td>17.8</td>
<td>17.4</td>
<td>15.5</td>
</tr>
</tbody>
</table>

**8.3. CROSS REACTIVITY**

A panel of 38 sera with potential interfering substances like antinuclear antibodies (n=10), rheumatoid factor (n=9), heterophilic antibodies (n=9) as well as patients with myeloma (n=10) were tested with the Platelia™ Dengue NS1 Ag. Another panel of 162 sera from patients with confirmed diseases other than dengue (West Nile, Yellow fever, CMV, HSV, VZV, etc...) was tested. All 200 samples were found to be negative with the Platelia™ Dengue NS1 Ag assay.

**9- LIMITATIONS OF THE PROCEDURE**

Diagnosis of recent infection by Dengue virus can only be established on the basis of a combination of clinical and biological datas. The result obtained on a single sample does not constitute a sufficient proof for diagnostic of recent infection.

**10- QUALITY CONTROL OF THE MANUFACTURER**

All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to the final commercialization of the product. Each lot is submitted to quality control assessments and is only released to the market, after conforming to pre-defined acceptance criteria. The records relating to production and control of each single lot are kept within Bio-Rad.
11- REFERENCES


This product contains human or animal components. Handle with care.
Danger
Causes severe skin burns and eye damage. May cause an allergic skin reaction.

Wear protective gloves/protective clothing/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. If skin irritation or rash occurs: Get medical advice/attention. Dispose of contents/container in accordance with local/regional/national/international regulations.

Peligro
Provoca quemaduras graves en la piel y lesiones oculares graves. Puede provocar una reacción alérgica en la piel.
Llevar guantes que aislen del frío/gafas/máscara.

EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos.

Quitar las lentes de contacto, si lleva y resulta fácil.

Seguir aclarando. EN CASO DE INGESTIÓN: Enjuagar la boca. NO provocar el vómito. EN CASO DE CONTACTO CON LA PIEL (o el pelo): Quitarse inmediatamente las prendas contaminadas.

Aclarar la piel con agua o ducharse. En caso de irritación o erupción cutánea: Consultar a un médico.

Eliminar el contenido o el recipiente conforme a la reglamentación local/regional/nacional/internacional.

(VI)
Vaara
Voimakasti ihoa syövyttävää ja silmiä vaurioittava. Voi aiheuttaa allergisen ihoreaktion.


(FI)
Vaara
Voimakasti ihoa syövyttävää ja silmiä vaurioittava. Voi aiheuttaa allergisen ihoreaktion.


(FR)
Danger
Provoque des brûlures de la peau et des lésions oculaires graves. Peut provoquer une allergie cutanée.


(HR)
Opasnost
Uzrokuje teške opekline kože i ozljede oka. Može izazvati alergijsku reakciju na koži.


(HU)
Veszély
Smarkiai nudegina odą ir pažeidžia akis. Allergiás bőrreakciót válthat ki.


(IT)
Pericolo
Provoca gravi ustioni cutanee e gravi lesioni oculari. Può provocare una reazione allergica cutanea.

**PT**

**Perigo**

Procura queimaduras nas pele e lesões oculares graves. Pode provocar uma reação alérgica cutânea.


**NO**

**Fare**

Nevarno
Povzroča hude opekline kože in poškodbe oči.
Lahko povzroči alergijski odziv kože.

Nebezpečenstvo
Provoacă arsuri grave ale pielii şi lezarea ochilor.
Môže vyvolat’ alergickú kožnú reakciu.