Leptospira IgM ELISA

Enzyme immunoassay for the qualitative determination of IgM class antibodies against Leptospira spp. in human serum or plasma (heparin).

REF  RE58941

Σ  96

2-8°C

U.S.: For research use only.
Not for use in diagnostic procedures.
1. INTENDED USE

The Leptospira IgM-ELISA is intended for the qualitative determination of IgM class antibodies against Leptospira spp. in human serum or plasma (heparin).
For research use only. Not for use in diagnostic procedures.

2. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of IgM-class antibodies against Leptospira spp. is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiter strip wells are coated with Leptospira antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled anti-human IgM conjugate is added. This conjugate binds to the captured Leptospira-specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of Leptospira-specific IgM antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

3. MATERIALS

3.1. Reagents supplied

- **Leptospira Coated Wells (IgM):** 12 break-apart 8-well snap-off strips coated with Leptospira antigens; in resealable aluminium foil.
- **IgM Sample Diluent:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; anti-human IgG; coloured green; ready to use; white cap; < 0.1 % MIT; < 0.1 % CMIT; < 0.1 % NaN₃.
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Solution (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap; < 1 % Ethanol; < 0.5 % Bromonitrodioxane.
- **Leptospira anti-IgM Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgM in phosphate buffer (10 mM); coloured red; ready to use; black cap; < 1 % Ethanol; < 0.5 % Bromonitrodioxane.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Leptospira IgM Positive Control:** 1 vial containing 2 mL control (human serum or plasma); coloured yellow; ready to use; red cap; < 0.1 % Bromonitrodioxan; < 0.1 % MIT.
- **Leptospira IgM Cut-off Control:** 1 vial containing 3 mL control (human serum or plasma); coloured yellow; ready to use; green cap; < 0.1 % Bromonitrodioxan; < 0.1 % MIT.
- **Leptospira IgM Negative Control:** 1 vial containing 2 mL control (human serum or plasma); coloured yellow; ready to use; blue cap; < 0.1 % MIT; < 0.1 % CMIT; < 0.1 % NaN₃.

3.2. Materials supplied

- 1 Strip holder
- 1 Cover foil
- 1 Test protocol

3.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
4. STABILITY AND STORAGE

Store the kit at 2 - 8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2 - 8 °C.

5. REAGENT PREPARATION

It is very important to bring all reagents, samples and standards/controls to room temperature (20 - 25 °C) before starting the test run!

5.1. Coated snap-off strips

The ready to use break-apart snap-off strips are coated with Leptospira antigen. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2 - 8 °C.

5.2. Washing Solution (20x conc.)

Dilute Washing Solution 1 + 19; e. g. 10 mL Washing Solution + 190 mL fresh and germ free redistilled water. The diluted buffer is stable for 5 days at room temperature. Crystals in the concentrate disappear by warming up to 37 °C in a water bath.

5.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2 - 8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

5.4. IgM Sample Diluent

The solution contains anti-human IgG class antibodies to eliminate competitive inhibition from specific IgG class antibodies and to remove rheumatoid factors.

6. SPECIMEN COLLECTION AND PREPARATION

Use human serum or plasma (heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the specimens should be kept at 2 - 8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing. Heat inactivation of samples is not recommended.

6.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgM Sample Diluent. Dispense 10 µL sample and 1 mL IgM Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.
7. **ASSAY PROCEDURE**

### 7.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of Washing Solution from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all specimens and standards/controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

- 1 well (e. g. A1) for the Substrate Blank,
- 1 well (e. g. B1) for the Negative Control,
- 2 wells (e. g. C1+D1) for the Cut-off Control and
- 1 well (e. g. E1) for the Positive Control

It is recommended to determine standards/controls and samples in duplicate.

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.

5. Dispense 100 µL Leptospira anti-IgM Conjugate into all wells except for the Substrate Blank well (e. g. A1). Cover with foil.
6. **Incubate for 30 min at room temperature.** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20 - 25 °C) in the dark.**
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate.

   Any blue colour developed during the incubation turns into yellow.
11. Measure the absorbance of the specimen at 450/620 nm within 30 min after addition of the Stop Solution.

### 7.2. Measurement

Adjust the ELISA Microwell Plate Reader to zero using the Substrate Blank in well A1.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the Substrate Blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

**Measure the absorbance** of all wells at 450 nm and record the absorbance values for each standard/control and sample in the distribution and identification plan.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

Where applicable calculate the **mean absorbance values** of all duplicates.
8. RESULTS

8.1. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate Blank** in A1: Absorbance value < 0.100
- **Negative Control** in B1: Absorbance value < 0.200 and < Cut-off
- **Cut-off Control** in C1 and D1: Absorbance value 0.150 – 1.300
- **Positive Control** in E1: Absorbance value > Cut-off

If these criteria are not met, the test is not valid and must be repeated.

8.2. Results in Units [U]

Samples (mean) absorbance value x 10 = [Units = U]

Example: \( \frac{1.591 \times 10}{0.43} = 37 \text{ U (Units)} \)

9. SPECIFIC PERFORMANCE CHARACTERISTICS

9.1. Precision

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (OD)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample #1</td>
<td>0.478</td>
<td>1.9</td>
</tr>
<tr>
<td>Sample #2</td>
<td>0.893</td>
<td>1.8</td>
</tr>
<tr>
<td>Sample #3</td>
<td>0.448</td>
<td>1.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (U)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample #1</td>
<td>19.15</td>
<td>3.2</td>
</tr>
<tr>
<td>Sample #2</td>
<td>10.10</td>
<td>4.2</td>
</tr>
</tbody>
</table>

9.2. Interferences

Interferences with hemolytic, lipemic or icteric specimen are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

9.3. Cross Reactivity

It cannot be excluded that Cytomegalovirus, Treponema pallidum and Coxiella specimens may result in false-positive IgM antibody results. In addition, it should be noted that IgM class antibodies directed against Leptospira generally remain detectable for months or even years but at low titer.

Note: The results refer to the groups of samples investigated; these are not guaranteed specifications.

10. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.
11. PRECAUTIONS AND WARNINGS

- For research use only. Not for use in diagnostic procedures.
- All materials should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense reagents without splashing accurately to the bottom of wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.
- The concentrations of the hazardous materials mentioned in point 4.1. are very low. Therefore there is hardly any toxicological risk. Nevertheless rinse with plenty of water upon contact with eyes, skin or mucous membranes and consult a doctor in case of irritations. All solutions should be handled with adequate care.

11.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.
ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIT</td>
<td>2-Methyl-2H-isothiazol-3-one</td>
</tr>
<tr>
<td>CMIT</td>
<td>5-Chloro-2-methyl-2H-isothiazol-3-one</td>
</tr>
<tr>
<td>NaN₃</td>
<td>Sodium azide</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Hydrogen peroxide</td>
</tr>
</tbody>
</table>

SCHEME OF THE ASSAY
Leptospira IgM ELISA

Test Preparation

Prepare reagents and samples as described. Establish the distribution and identification plan for all specimens and controls on the result sheet. Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

<table>
<thead>
<tr>
<th></th>
<th>Substrate Blank (e.g. A1)</th>
<th>Negative Control</th>
<th>Cut-off Control</th>
<th>Positive Control</th>
<th>Sample (diluted 1+100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>-</td>
<td>100 µL</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cut-off Control</td>
<td>-</td>
<td>-</td>
<td>100 µL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Positive Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100 µL</td>
<td>-</td>
</tr>
<tr>
<td>Sample (diluted 1+100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

Cover wells with foil supplied in the kit
**Incubate for 1 h at 37 °C**
Wash each well three times with 300 µL of Washing Solution

<table>
<thead>
<tr>
<th></th>
<th>-</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjugate</td>
<td>-</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

Cover wells with foil supplied in the kit
**Incubate for 30 min at room temperature**
Wash each well three times with 300 µL of Washing Solution

<table>
<thead>
<tr>
<th></th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMB Substrate</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

**Incubate for exactly 15 min at room temperature in the dark**

<table>
<thead>
<tr>
<th></th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stop Solution</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

Photometric measurement at 450 nm (reference wavelength: 620 nm)
Symbols / Symbole / Symbôles / Símbolos / Σύμβολα

<table>
<thead>
<tr>
<th>REF</th>
<th>Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT</td>
<td>Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:</td>
</tr>
<tr>
<td>Σύμβολα</td>
<td>Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:</td>
</tr>
<tr>
<td>Σύμβολα</td>
<td>Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα</td>
</tr>
<tr>
<td>Σύμβολα</td>
<td>Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο</td>
</tr>
<tr>
<td>Σύμβολα</td>
<td>In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.</td>
</tr>
<tr>
<td>Σύμβολα</td>
<td>Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluación. / Kit de evaluación. / Kit di valutazione. / Κιτ Αξιολόγησης.</td>
</tr>
<tr>
<td>Σύμβολα</td>
<td>Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Leggere le istruzioni prima dell’uso. / Διαβάστε τις οδηγίες πριν την χρήση.</td>
</tr>
<tr>
<td>Σύμβολα</td>
<td>Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.</td>
</tr>
<tr>
<td>Σύμβολα</td>
<td>Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:</td>
</tr>
<tr>
<td>Σύμβολα</td>
<td>Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:</td>
</tr>
<tr>
<td>Σύμβολα</td>
<td>Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!</td>
</tr>
</tbody>
</table>

Symbols of the kit components see MATERIALS SUPPLIED.

Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.

Voir MATERIEL FOURNI pour les symbôles des composants du kit.

Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.

Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.

Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.

Για τα σύμβολα των συστατικών του κιτ συμβουλεύετε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER’S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

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