Leptospira IgG ELISA

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

REF RE58931
∑ 96

EU: IVD CE
U.S.: For research use only.
Not for use in diagnostic procedures.
1. INTRODUCTION

Leptospirosis (also known as Weil's syndrome) is probably the most widespread zoonosis in the world. It is caused by infection with spirochete bacteria of the genus Leptospira and affects humans as well as a broad spectrum of animal hosts. The incidence is significantly higher in warm climate countries than in temperate regions. The disease is seasonal, with peak incidence occurring in summer or fall in temperate regions, where temperature is the limiting factor in survival of leptospires, and during rainy seasons in warm climate regions, where rapid desiccation would otherwise prevent survival.

Natural reservoirs for the pathogenic Leptospira interrogans include rodents as well as a large variety of domesticated mammals (e.g. pigs, cattle and dogs). Leptospires occupy the lumen of nephritic tubules in their natural host and are shed into the urine.

Transmission can occur when humans are directly or indirectly exposed to the urine of infected animals or a urine-polluted environment. Leptospires gain entry into the human blood stream via cuts, skin abrasions or mucous membranes through contact with moist soil, vegetation, and contaminated waters; handling infected animal tissues; and ingestion of food and water. Leptospires are rarely transmitted from human to human.

The incubation period is usually 5-14 days, with a range of 2-30 days.

The spectrum of clinical symptoms is extremely wide. The vast majority of leptospiiral infections are either subclinical or result in very mild illness and recover without any complications. Clinical manifestations of leptospirosis range from mild influenza-like symptoms to severe life-threatening disease forms, characterized by jaundice, renal failure, bleeding and severe pulmonary hemorrhage.

The clinical presentation of leptospirosis is biphasic, with the acute or septicemic phase lasting about a week, followed by the immune phase, characterized by antibody production and excretion of leptospires in the urine. Most of the complications of leptospirosis are associated with localization of leptospires within the tissues during the immune phase and thus occur during the second week of the illness. The classical syndrome of Weil's disease represents only the most severe presentation. It is characterized by jaundice, renal failure, hemorrhage and myocarditis with arrhythmias.

<table>
<thead>
<tr>
<th>Species</th>
<th>Disease</th>
<th>Symptoms</th>
<th>Mechanism of Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptospira</td>
<td>Leptospirosis</td>
<td>Wide spectrum of clinical symptoms: mild influenza-like symptoms to severe life-threatening disease forms</td>
<td>direct or indirect contact with the urine of an infected animal (via cuts, skin abrasions or mucous membranes)</td>
</tr>
<tr>
<td>spp.</td>
<td>Weil’s disease</td>
<td>jaundice, renal failure, haemorrhage and myocarditis with arrhythmias</td>
<td></td>
</tr>
</tbody>
</table>

The presence of pathogen resp. infection may be identified by

- Pathogen detection: dark-field microscopy
  - culture from blood, urine, cerebrospinal fluid or tissues
  - PCR
- Serology: microscopic agglutination test (MAT), ELISA

2. INTENDED USE

The Leptospira IgG-ELISA is intended for the qualitative determination of IgG class antibodies against Leptospira spp. in human serum or plasma (heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of IgG-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 IgG 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 IgG 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.
4. MATERIALS

4.1. Reagents supplied

- **Leptospira Coated Wells (IgG):** 12 break-apart 8-well snap-off strips coated with Leptospira antigens; in resealable aluminium foil.
- **IgG Sample Diluent:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; 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-IgG Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap; < 1 % Ethanol; < 0.5 % Bromonitrodioxane.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.04 %; ready to use; yellow cap; < 0.0001 % CMIT; < 0.0001 % MIT; < 0.01 % H₂O₂.
- **Leptospira IgG Positive Control:** 1 vial containing 2 mL control (human serum or plasma); coloured yellow; ready to use; red cap; < 0.1 % Bromonitrodioxane; < 0.1 % MIT.
- **Leptospira IgG Cut-off Control:** 1 vial containing 3 mL control (human serum or plasma); coloured yellow; ready to use; green cap; < 0.1 % Bromonitrodioxane; < 0.1 % MIT.
- **Leptospira IgG 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.

4.2. Materials supplied

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

4.3. 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

5. 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.

6. 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!

6.1. 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.

6.2. 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.
6.3. 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.

7. 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.

7.1. Sample Dilution
Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10 µL sample and 1 mL IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

8.1. 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 patient 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-IgG 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.

8.2. 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 patient 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.

9. RESULTS

9.1. 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.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43

Cut-off = 0.43

9.2.1. Results in Units [U]

Patient (mean) absorbance value x 10 = [Units = U] / Cut-off

Example: 1.591 x 10 = 37 U (Units)

9.3. Interpretation of Results

<table>
<thead>
<tr>
<th>Cut-off</th>
<th>10 U</th>
</tr>
</thead>
</table>
| Positive | > 11 U | Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
| Equivocal| 9 – 11 U | Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as negative.
| Negative | < 9 U | The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.

Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.
10. SPECIFIC PERFORMANCE CHARACTERISTICS

10.1. Precision

<table>
<thead>
<tr>
<th>Intraassay</th>
<th>n</th>
<th>Mean (OD)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample #1</td>
<td>23</td>
<td>0.437</td>
<td>3.2</td>
</tr>
<tr>
<td>Sample #2</td>
<td>24</td>
<td>0.994</td>
<td>2.4</td>
</tr>
<tr>
<td>Sample #3</td>
<td>24</td>
<td>0.544</td>
<td>2.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interassay</th>
<th>n</th>
<th>Mean (U)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample #1</td>
<td>12</td>
<td>21.97</td>
<td>8.2</td>
</tr>
<tr>
<td>Sample #2</td>
<td>12</td>
<td>13.10</td>
<td>3.5</td>
</tr>
</tbody>
</table>

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.

It is 97.4 %.

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

It is > 98 %.

10.4. 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.

10.5. Cross Reactivity

It cannot be excluded that polyclonal B-cell activation induced by Epstein-Barr virus (EBV) or the presence of Rheumatoid Factors may result in false-positive Leptospira IgG antibody results.

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

11. LIMITATIONS OF THE PROCEDURE

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

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- 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 patient 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.

12.1. 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.
BIBLIOGRAPHY


Johnson, R. C. (2001) Leptospira. In: Medical Microbiology. edited by Baron, S. The University of Texas Medical Branch


ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</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>NaN</td>
<td>Sodium azide</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Hydrogen peroxide</td>
</tr>
</tbody>
</table>
**SCHEME OF THE ASSAY**  
Leptospira IgG 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>Conjugate</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
</tr>
</thead>
</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>TMB Substrate</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
</tr>
</thead>
</table>

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

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

Photometric measurement at 450 nm  
(reference wavelength: 620 nm)
LIABILITY: Complaints will be accepted in each mode – written or vocal. Preferred is that the complaint is accompanied with the test performance and results. 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. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.

Symbols Version 3.5 / 2012-01-20