Revised 7 April 2014 rm (Vers. 4.1)

IVD

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

The Leptospira IgG ELISA test is an enzyme immunoassay for the detection of antibodies to Leptospira biflexa (serovar patoc 1) for the serological confirmation of infections in serum and plasma.

This test is intended to be performed by trained laboratory personnel only.

2 SUMMARY AND EXPLANATION

The clinical manifestations of leptospirosis range from a mild catarrh-like illness to icteric disease with severe liver and kidney involvement. Natural reservoirs for leptospirosis include rodents as well as a large variety of domesticated mammals. The organisms occupy the lumen of nephritic tubules in their natural host and are shed into the urine. Human infection derives from direct exposure to infected animals (veterinarians, abattoir workers, or dairy workers for example) or by exposure to environments contaminated by animal carriers (e.g. agricultural workers). Bathing or swimming in water sources about which livestock have been pastured has been demonstrated to be a potential infection hazard. The organisms enter the host through skin abrasions, mucosal surfaces or the eye. The incubation period can range from 3 to 30 days but is usually found to be 10 to 12 days. Antibodies can become detectable by the 6^{th} to 10^{th} day of disease and generally reach peak levels within 3 to 4 weeks. Antibody levels then gradually recede but may remain detectable for years.

Epidemiologic factors, clinical findings, exposure in endemic regions and other laboratory results should be considered in diagnosing acute disease. Acute disease diagnosis will also include a positive laboratory confirmation in many cases.

This test is designed to measure acute infections with leptospira. Confirmation of a positive sample by additional methods should be followed.

3 ASSAY PRINCIPLE

The microwells are coated with purified Leptospira Patoc 1 antigen. During the first incubation with the diluted patient sera, any antibodies which are reactive with the antigen will bind to the coated wells. After washing to remove the rest of the sample, the Enzyme Conjugate is added. If antibodies have been bound to the wells, the Enzyme Conjugate will then bind to these antibodies. After another series of washes, a chromogen (tetramethylbenzidine, or TMB) is added. If the Enzyme Conjugate is present, the peroxidase will catalyze a reaction that consumes the peroxide and turns the chromogen from clear to blue. Addition of the Stop Solution ends the reaction and turns the blue color to a bright yellow color.

The reaction may then be read visually or with an ELISA reader.

CE

Revised 7 April 2014 rm (Vers. 4.1)

IVD

4 REAGENTS

Item	Description	Symbol
Test Strips	Microwells containing Leptospira antigen - 96 test wells in a test strip holder.	MPS 12x8
Enzyme Conjugate	One (1) bottle containing 11 mL of anti-human IgG antibody conjugated to peroxidase.	CONJ
Positive Control	One (1) vial containing 1 mL of diluted positive control.	CONTROL +
Negative Control	One (1) vial containing 1 mL of diluted human serum	CONTROL –
Chromogen	One (1) bottle containing 11 mL of the chromogen tetramethylbenzidine (TMB).	SUBS TMB
Wash Concentrate (20X)	One (1) bottle containing 25 mL of concentrated buffer and surfactant.	WASHB·20x
Dilution Buffer	Two (2) bottles containing 30 mL of buffered protein solution.	DIL
Stop Solution	One (1) bottle containing 11 mL of 0.73 M phosphoric acid.	STOP

5 STATEMENT OF WARNINGS

- **Do not deviate from the specified procedures when performing this assay**. All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance characteristics. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
- For In Vitro Diagnostic Use Only.
- Do not use reagents that are beyond their expiration dates. Expiration dates are on each reagent label. Use of reagents beyond their expiration dates may affect results.
- Unused microwells should be stored in the desiccated pouch to protect them from moisture.
- Do not use solutions if they precipitate or become cloudy.
  Exception: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.
- Do not add azides to the samples or any of the reagents.
- Controls and some reagents contain thimerosal as a preservative, which may be irritating to skin, eyes and mucous membranes. In case of contact, flush eyes or rinse skin with copious amounts of water.
- Do not use serum that may have supported microbial growth, or is cloudy due to high lipid content. Samples high in lipids should be clarified before use.
- Treat all reagents and samples as potentially infectious materials. Negative control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV by required test methods. Use care to prevent aerosols and decontaminate any spills of samples.
- Stop solution is a 5% solution of phosphoric acid in water. If spilled on the skin, wash with copious amounts of water. If acid gets into the eyes, wash with copious amounts of water and seek medical attention.

- Persons who are color blind or visually impaired may not be able to read the test visually and should use spectrophotometric readings to interpret results.
- Use separate pipette tips for each sample and reagent to avoid cross contamination
- Reagents should be inspected for evidence of bacterial or fungal contamination
- Do not reuse microwells
- All components in this kit have been standardized as a unit. Do not intermix components from different kit lots or other manufacturers kits

6 STORAGE

Reagents, strips and bottled components should be stored at 2 °C - 8 °C

Squeeze bottle containing diluted wash buffer may be stored at room temperature (15 °C - 25 °C)

7 PREPARATION

Before use, bring all reagents and samples to room temperature (15 °C - 25 °C) and mix.

(20X) Wash Concentrate
may precipitate during refrigerated storage, but will go back into solution when brought to room temperature and mixed. **Ensure that (20X) Wash Concentrate is completely in solution before diluting to working concentration.**

To dilute (20X) wash concentrate to working dilution, remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 mL of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.

8 SPECIMEN COLLECTION AND HANDLING

The Leptospira IgG ELISA test should be performed on serum or plasma.

Serological Specimens should be collected under aseptic conditions. Hemolysis is avoided through prompt separation of the serum from the clot.
Serum may be stored at 2 °C - 8 °C for up to five days. Serum may be frozen below -20 °C for 3-6 months.
Do not heat inactivate samples and avoid repeated freezing and thawing of samples. Lipemic and strongly hemolytic serum should be avoided.

Single specimens are used to assess exposure; two specimens collected at different times from the same individual are used to show sero-conversion. **Paired specimens should be tested at the same time**. It is recommended that a convalescent specimen be collected from patients showing either an initially non-reactive result or a weakly reactive result.

Revised 7 April 2014 rm (Vers. 4.1)

IVD

9 PROCEDURE

9.1 Materials Provided

Leptospira IgG Microwell ELISA Kit

9.2 Materials Required But Not Provided

- Micropipette
- Squeeze bottle for washing strips (narrow tip is recommended)
- Reagent grade (DI) water
- Graduated Cylinder
- Sample Dilution Tubes
- Absorbent paper
- Timer

9.3 Suggested Materials

ELISA plate reader with a 450 nm and a 620-650 nm filter (optional if results are read visually)

9.4 Proper Temperature

All incubations are at room temperature (15 °C - 25 °C)

9.5 Test Procedure

Notes:

- Ensure all samples and reagents are at room temperature (15 °C - 25 °C)
- When running the assay, try to avoid the formation of bubbles in the wells. Bubbles may affect overall performance and reading of end results. Slapping the wells out on a clean absorbent towel after each step should help to minimize bubbles in the wells.
- Negative and positive controls are supplied pre-diluted. DO NOT dilute further.

1. Break off number of wells needed (two for controls plus number of samples and one for blank, if used) and place in strip holder.
2. ***Dilute patient sera 1:40 in Dilution Buffer*** (e.g. 10 µL sera and 390 µL dilution buffer).

   Add **100 µL** of negative control to well #1,
   100 µL of positive control to well #2 and
   100 µL of the samples to the remaining wells.
3. Incubate at room temperature for **10 minutes**, then wash.* After last wash step, slap the wells on a clean absorbent towel to remove excess wash buffer.

4. Add **2 drops (100 µL)** of Enzyme Conjugate to each well.
5. Incubate at room temperature for **10 minutes**, then wash.* After last wash step, slap the wells on a clean absorbent towel to remove excess wash buffer.
6. Add **2 drops (100 µL)** of the Chromogen to each well.
7. Incubate at room temperature for **5 minutes**.
8. Add **2 drops (100 µL)** of the Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger for approximately **15 seconds**.
9. Read within one hour of adding Stop Solution.

* Washings consist of vigorously filling each well to overflowing and decanting contents three (3) separate times. When possible, avoid formation of bubbles in the wells as this may affect the end results.

10 READING RESULTS

Visually:

Look at each well against a white background (e.g. paper towel) and record as clear or +, ++ or +++ reaction.

ELISA Reader:

Zero reader on air or blank well, using the dilution buffer as the sample.
Set for bichromatic readings at 450/620-650 nm.

11 QUALITY CONTROL

The use of controls allows validation of kit stability. The kit should not be used if any of the controls are out of range.

Expected values for the controls are:

Negative - 0.0 to 0.3 OD units

Positive - 0.5 OD units and above

12 TROUBLESHOOTING

Negative control has excessive color after development.

Reason: inadequate washings

Correction: wash more vigorously. Remove excessive liquid from the wells by tapping against an Absorbent towel.
Do not allow test wells to dry out.

13 INTERPRETATION OF THE TEST

Initially Non-reactive:
Samples interpreted as non-reactive (0.0 - 0.3 OD units, or zero color) indicate antibody is not present in the sample. Since antibody may not be present during early disease, (5 - 8 days incubation), confirmation 2 - 3 weeks later is indicated for laboratory diagnosis.

At this later time, patients showing weak reactions (0.3 - 1.0 OD or +, ++) should be further tested by alternate methods or re-tested 10-14 days later. A convalescent serum with a significant reaction (>1.0 OD) indicates the formation of specific antibody against leptospira. An initially negative result followed by a positive result implies seroconversion.

Initially Weakly Reactive:
Weakly reactive specimens should be cautiously interpreted. In normal populations, weakly reactive samples are infrequent but possible. Confirmation using a sample collected 2-3 weeks later (paired acute and convalescent sera) is recommended. >1.0 OD in the second sample confirms the presence of recent, specific antibody. [Caution: If this is a cross-reactive antibody, the convalescent serum sample may not show a higher antibody level than the acute sample.] If sample reading remains at 0.3 - 1.0 OD, or +, ++, a second methodology should be considered, or the sample may be interpreted as taken beyond rising titer (titer declining).

Initially Reactive:
Samples interpreted as strongly reactive (>1.0 OD or +++) may indicate the presence of specific antibody. Antibody presence alone cannot be used for diagnosis of acute infection, since antibodies from prior exposure may circulate for a prolonged period of time.

14 LIMITATIONS OF THE PROCEDURE

Serologic results are an aid in diagnosis but cannot be used as the sole method of diagnosis.

The ELISA has been tested against many serovars, but cannot guarantee that all strains will react equally.

Do not use in veterinary samples.

Revised 7 April 2014 rm (Vers. 4.1)

IVD

Treatment is often indicated prior to completion of serologic diagnosis, which requires at least two weeks. Acute leptospirosis must be treated immediately and should not wait for serological confirmation. Diagnosis of leptospira infection should not be made based on results of the ELISA test alone, but in conjunction with other clinical signs and symptoms and other laboratory findings.
Epidemiologic factors, clinical findings, exposure to endemic regions, and other laboratory results should be considered when making a diagnosis.

Many strains and serovars of leptospira are known. Many of the strains are geographically dominant in some areas and not in others. Biflexa Patoc 1 is known to cross react with most serovars **but usually does not cross-react with animal strains.** The relative strength of the reactions may vary by antigen. This must be considered during interpretation. Use of culture or the MAT test is recommended for confirmation as these test are serovar specific.

Since serological assay methods may yield different results for weakly reactive samples, a second serological method (i.e. an alternative method that separately identifies IgM and IgG antibody) is recommended.

15 EXPECTED VALUES

The number of antibody positive subjects in a population depends on two factors: disease prevalence and clinical criteria used to select the tested population. Because very few positives should be seen in a randomly screened population in a non-endemic area, most serology tests are not specific enough to screen non-endemic populations. Even in an endemic region, serology screening often yields many false positives if used to randomly screen patients. Serology tests are useful to test patients in an endemic region with signs and symptoms consistent with the disease.

Antibody levels are generally low or absent during very early infection. Symptomatic patients may have no antibody during the first 1-2 weeks after exposure and the antibody titer will rise with time.

16 PERFORMANCE CHARACTERISTICS

		Reference Method*	
		+	-
EIA-4245	+	8	4
	-	2	28

Positive Agreement: 80% (8/10)

Negative Agreement: 87.5% (28/32)

Revised 7 April 2014 rm (Vers. 4.1)

IVD

*Reference Method refers to a commercially available ELISA.

17 REFERENCES

1. Kelly PW. Leptospirosis. In, Infectious disease in medicine and surgery. Gorbach S, Bartlett J, Balcklow N, (eds): Philadelphia, Saunders, 1991, pp.1295-1302.
2. Ribeiro MA, Assis CSN, Romero EC. Serodiagnosis of human leptospirosis employing immunodominant antigen.
   Serodiagn. Immunother. Infect. Disease 1994; 6:140-144.
3. Turner LH. Leptospirosis II.
   Trans. Royal Soc. Trop. Med. & Hygiene 1968; 62:880-889.
4. Pappas MG, Ballou WR, Gray MR, et. al. Rapid serodiagnosis of leptospirosis using the IgM-specific dot-ELISA: Comparison with the microscopic agglutination test.
   Am J Trop Med Hyg 1985; 34(2):346-354.
5. Alder B, Murphy AM, Locarnini SA, et.al. Detection of specific anti-leptospiral immunoglobulin M and immunoglobulin G in human serum by solid phase enzyme-linked immunosorbent assay.
   J. Clin. Micro 1980; 11:452-457.
6. Cinco M, Balanzini D, Banfi E. Evaluation of an immunoenzymatic test (ELISA) for the diagnosis of leptospirosis in Italy.
   Eur. J. Epidemiology 1992; 8:677-682.
7. Ribeiro MA, Sousa CC, Almeida SHP. Dot-ELISA for human leptospirosis employing immunodominant antigen.
   J. Trop.Med. Hyg. 1995; 98:452-456.
8. Gussenhoven GC, van der Hoorn M, Goris M, et. al. LEPTO dipstick, a dipstick assay for detection of leptospira-specific immunoglobulin M antibodies in human sera.
   J. Clin. Micro. 1997; 35(1):92-97.
9. Watt G, Alquiza LM, Padre LP, et. al. The rapid diagnosis of leptospirosis: A prospective comparison of the dot enzyme-linked immunosorbent assay and the genus-specific microscopic agglutination test at different stages of illness.
   J. Infect. Dis. 1988; 157(4)840-842.
10. Levett PN, Branch SL, Paxton H. Prospective Evaluation of Dot-ELISA Method for Detection of Acute Leptospirosis. Abstract of the 37th ICAAC (ASM) 1997; D-4: 83.

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