Leptospira Antibody IgM GENLISA™ ELISA

REF : KBD951

Ver 1.3

IVD

Enzyme Immunoassay for Qualitative Determination of Leptospira Antibody IgM in human serum and plasma.

IVD	**For In-vitro Diagnostic Use**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

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CE **REF** KBD951 Σ 96 tests

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Introduction:

Leptospirosis is considered an emerging zoonotic disease of global importance and is found in both urban and rural areas of affected countries. It is caused by the genus *Leptospira. which* are motile spirochaetes of which there are 16 genomo-species as delineated by DNA–DNA hybridization. Transmission to humans occurs through penetration of the organism into the blood stream via cuts, skin abrasions or mucus membranes. Infection may range in presentation from subclinical to severe . Onset is usually sudden, with symptoms including headache, fever, muscle pains, conjunctival infection, meningitis and abdominal pains. Severe complications such as hepato-renal failure and central nervous system involvement may arise. It is also known as 'Weil's syndrome.'

Intended Use:

The Leptospira Antibody IgM GENLISA™ ELISA is intended for the qualitative determination of IgM class antibodies in human serum and plasma.

Principle:

Leptospira Antibody IgM GENLISA™ ELISA is an indirect enzyme linked immunosorbent assay for qualitative determination of IgM antibody present in the human serum and plasma. Antigens are pre-coated onto microwells. Samples, Controls are pipetted into microwells and Leptospira antibody present in sample binds to the antigen coated on the wells. Enzyme conjugate is pipetted and incubated to form an immune complex. After washing microwells in order to remove any non-specific binding, the substrate solution is added to microwells and color develops proportionally to the amount of Leptospira Antibody present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Leptospira Antigen Coated Microtiter Plate (12 x 8wells) - 1 no
2. Negative Control – 0.5 ml
3. Positive Control concentrated – 50ul
4. Anti-Human IgM:HRP Conjugate - 12 ml
5. Positive Control diluent – 3ml
6. (20X) Wash Buffer - 25 ml
7. Sample Diluent - 50 ml
8. TMB Substrate - 12 ml
9. Stop Solution - 12 ml
10. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 5 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. Once we receive the positive control concentrated vial, Immediately make aliquots and freeze at -20°C. Whenever required remove an aliquot, thaw it dilute it accordingly and ten run the ELISA.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Serum- Coagulate at room temperature for 10 - 20 minutes; centrifuge for 20 minutes at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge the sample.

Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10 - 20 minutes; centrifuge for 15 minutes at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, re centrifuge.

Sample Dilution:
To make 1:100 Dilution, dilute 5 ul Sample + 495 ul Sample Diluent.

Reagent Preparation:

1. To make Wash Buffer (1X); dilute 25 ml of 20X Wash Buffer in 475 ml of DI water. This is the working solution.
2. Positive control preparation – Dilute the concentrated positive control i.e. 6ul positive control concentrated + 24ul of positive control diluent to prepare 30ul of positive control solution.
3. Allow all components to reach RT (Room Temperature) prior to use in the assay.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **25 ul Control, Diluted Sample** in appropriate wells.
3. Seal the plate and Incubate at 37°C for 60 minutes.
4. Aspirate and wash plate 5 times with **(1x) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
5. Add **100 ul** of **Anti- Human IgM:HRP Conjugate** to each well. Incubate at 37°C for 30 minutes.
6. Repeat the Wash Step as mentioned in step 4.
7. Add **100 ul** of **TMB Substrate** into each well.
8. Incubate at RT for 30 minutes.
9. Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Calculation of Results:

Method 1
Calculate the cut-off manually to ensure each laboratory assay variance is accommodated. If you wish to run and interpret the results directly, you may use the Method 2 indicated herein below. Method 1 helps ensure the variation in the assays on personnel to personnel basis and on the laboratory floor level are incorporated for a higher degree of accuracy.

Cut Off (CO) Calculation:
Cut Off (CO) = Mean O.D. of Neg. Control + 0.2 **(cut off factor)**

Example:
Cut Off Value = O.D. of Neg. Control 1 + O.D. of Neg. Control 2 / 2 + 0.2
= 0.102 + 0.132 / 2 + 0.2
= 0.317

Reference Values:
Negative Results: Samples with O.D below or equal to the Cut Off Value (COV) are reported as Non-Reactive.
Equivocal Results: Samples with O.D above Cut Off Value (COV) and below or equal to O.D of 0.5 are reported as Equivocal / Mildly Positive
Positive Results: Samples with O.D above 0.5 are reported as Reactive.

Interpretation of Results:

Negative Value	Absorbance ≤ COV	No antibodies present against specific pathogen.
Equivocal / Mildly Positive	Absorbance > COV and ≤ 0.5	Equivocal Samples should be retested.
Positive Value	Absorbance > 0.5	Antibodies against specific pathogen are present.

Method 2
Alternately, the absorbance values calculated maybe used directly to interpret the results as under -

Cut-Off (CO) = 0.300

Reference Values:
Negative Results: Samples with O.D below or equal to the Cut Off Value (COV) are reported as Non-Reactive.
Equivocal Results: Samples with O.D above Cut Off Value (COV) and below or equal to O.D of 0.5 are reported as Equivocal / Mildly Positive
Positive Results: Samples with O.D above 0.5 are reported as Reactive.

Interpretation of Results:

Negative Value	Absorbance ≤ COV	No antibodies present against specific pathogen.
Equivocal / Mildly Positive	Absorbance > COV and ≤ 0.5	Equivocal Samples should be retested.
Positive Value	Absorbance > 0.5	Antibodies against specific pathogen are present.

Criteria of Validation:

Leptospira Antibody IgM results are considered to be valid, if

Negative Control	O.D < 0.2
Positive Control	O.D > 0.5

State Of Infection:

Serology	Significance
IgM	Primary Antibody Response. High IgM titer with Low IgG titer: Current or recent Infection. Persisting IgM: Rare.
IgG	Secondary Antibody Response. May persist for several years. High IgG titer with Low IgM titer: Indicates Past Infection.

Reference Values:

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too.

Limitations of Method:

Any clinical diagnosis should not be based on the results of in-vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

Safety Precautions:

- **This kit is For In-vitro Diagnostic Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Pudgala LLP.

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THANK YOU FOR USING KRISHGEN PRODUCT!

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EC REP

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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

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… However, a presumptive diagnosis of leptospirosis can be … have had leptospirosis can retain
high levels of IgM and IgG … of an IgM-ELISA and the MAT for diagnosis of acute leptospirosis …

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **25 ul Control, Diluted Sample** in appropriate wells
3	Seal the plate and Incubate at 37°C for 60 minutes.
4	Aspirate and **wash plate 5 times** with **(1x) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
5	Add **100 ul** of **Anti-Human IgM:HRP Conjugate** to each well.
6	Incubate at 37°C for 30 minutes.
7	Repeat the Wash Step as mentioned in step 4.
8	Add **100 ul** of **TMB Substrate** into each well.
9	**Incubate** at **RT** for **30 minutes**.
10	Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes stopping the reaction.

SYMBOLS KEY

MTP	Coated Microtiter Plate (12 x 8 wells)
CNTRL	Control
ENZY CONJ	Enzyme Conjugate
SAMP DIL	Sample Diluent
20x WASH BUF	(20x) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature