Brucella Antibody IgM GENLISA® ELISA

REF : KBD131

Ver 2.4

IVD

Enzyme Immunoassay for Qualitative Determination of Brucella 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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REF KBD131 Σ 96 tests

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Introduction:

Brucella is a gram-negative coccobacilli capable of infecting a wide range of animals including human. Of the three species causing human infection, *B. melitensis* is the most pathogenic followed by *B. suis* and *B. abortus*. Brucellosis is transmitted through contaminated and untreated milk and milk products and by direct contact with infected animals (cattle, sheep, goats, pigs, camels, buffaloes, and seals), animal carcasses, and abortion materials. Worldwide, millions of individuals are at risk, especially in developing countries where the infection in animals has not been brought under control, heat treatment procedures of milk (e.g. pasteurization) are not routinely applied, and food habits such as consumption of raw milk. The incubation period of brucellosis is usually one to three weeks, but sometimes may be several months. The illness may be mild and self-limiting or severe. The disease is accompanied by continued, intermittent, or irregular fever, headache, weight loss and generalized aching and fatigue. Urogenital symptoms may dominate the clinical presentation in some patients.
This method uses *B. abortus* outer membrane, which is shared by the other species. Brucella Antibody IgG and IgA antibodies persist for many years after infection. A significant increase in Brucella Antibody IgG level is in patients with symptoms of brucellosis is indicative of recent exposure. IgM antibodies are present in acute brucellosis and also found in about 33% of patients with chronic brucellosis.

Intended Use:

The Brucella Antibody IgM GENLISA® ELISA is intended for the qualitative determination of IgM antibodies to Brucella in human serum and plasma.

Principle:

Brucella Antibody IgM GENLISA® ELISA is an indirect enzyme linked immnunosorbent assay for qualitative determination of IgM antibody present in the human serum and plasma. Microtiter wells are pre-coated with brucella antigen. Samples and Controls are pipetted into microwells and Brucella Antibody IgM antibody present in sample binds to the antigen coated on the wells. Enzyme Conjugate antibody 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 Brucella Antibody IgM present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Brucella antigen Coated Microtiter Plate (96 wells) - 1 no
2. Positive control - 1 ml
3. Negative control - 1 ml
4. Calibrator – 1 ml
5. Enzyme conjugate - 12 ml
6. Sample diluent - 22 ml
7. (20X) Wash Buffer – 25 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. Deionized (DI) water.
3. Precision single and multi-channel pipette and disposable tips.
4. Disposable pipette tips.
5. Absorbent paper.
6. Graph 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. 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.
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, re-centrifuge.

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:
Prepare 1:21 dilution of test samples, by adding 10 ul of the sample to 200 ul of sample diluent. Mix well.

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. (1X) Wash Buffer Dilution: To make (1X) Wash Buffer, add 25ml of (20X) Wash Buffer to 475ml of DI water. This is the working solution.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. For the reagent blank, dispense **100 ul sample diluent** in 1A well position.
3. Add **100 ul Calibrators, Controls & diluted Sample** in appropriate wells.
4. Seal the plate and Incubate at Room Temperature for 20 minutes.
5. Aspirate and wash plate 3 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.
6. Add **100 ul** of **Enzyme Conjugate** to each well.
7. Incubate at Room Temperature for 20 minutes.
8. Repeat the Wash Step as mentioned in step 5.
9. Add **100 ul** of **TMB Substrate** into each well.
10. Incubate at Room Temperature for 10 minutes.
11. 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:

Cut off (CO) Calculation**:**

Cut off (CO) = **Calibrator OD x Calibrator Factor (CF)**

Note: The value of calibrator factor might vary from lot to lot.

Antibody Index Calculation:

Antibody Index: $\frac{\text{Absorbance of Sample}}{\text{Cut-off value.}}$

Example of typical results:

Mean Absorbance of Calibrator = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5 = 0.400
Positive Control OD = 1.2
Antibody Index of Positive Control = 1.2/0.4 = 3
Patient Sample OD = 1.6
Antibody Index of Sample = 1.6/0.4 = 4.0

Validity of the test:

The test run may be considered valid provided the following criteria are met:

1. The absorbance of the Calibrator should be greater than 0.250.
2. The Antibody Index for Negative control should be less than 0.9.
3. The Antibody Index for Positive control should fall within the range 2.1 – 3.8.

Interpretation of Results:

The following is intended as a guide to interpretation of Brucella Antibody IgM test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

If Antibody Index is,
<0.9 - No detectable antibody to Brucella Antibody IgM by ELISA
0.9-1.1 - Borderline positive. Follow-up testing is recommended if clinically indicated.
>1.1 - Detectable antibody to Brucella Antibody IgM by ELISA

Limitations of the test

1. The Test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Lipemic or hemolyzed samples may cause erroneous results.

Performance Characteristics:

Sensitivity and Specificity:
178 patient sera were tested by this Brucella Antibody IgM ELISA and a reference ELISA method. 26 sera were positive and 149 were negative by both methods (98% agreement). The results are summarized below:

		Brucella Antibody IgM ELISA		
		+	-	Total
Reference ELISA Kit	+	23	2	25
	-	1	152	153
	Total	24	154	178

Precision:
Intra-Assay:

Sample	No. of Replicates	Mean	SD	CV %
1	16	1.49	0.066	4.43
2	16	1.01	0.051	5.50
3	16	0.19	0.012	6.31

Inter-Assay:

Sample	No. of Replicates	Mean	SD	CV %
1	10	1.41	0.139	09.85
2	10	0.97	0.100	10.30
3	10	0.20	0.022	11.00

References:

1. Gad El-Rab MO; Kambal AM. Evaluation of a Brucella enzyme immunoassay test (ELISA) in comparison with bacteriological culture and agglutination. J Infect 1998; 36(2):197-201.
2. Mikolon AB; Gardner IA; Hietala SK; Hernandez de Anda J; Chamizo Pestana E; Hennager SG; Edmondson AJ. Evaluation of North American antibody detection tests for diagnosis of brucellosis in goats. J Clin Microbiol 1998; 36(6):1716-22.
3. Bowden RA; Cloeckaert A; Zygmunt MS; Bernard S; Dubray G. Surface exposure of outer membrane protein and lipopolysaccharide epitopes in Brucella species studied by enzyme-linked immunosorbent assay and flow cytometry. Infect Immun 1995; 63(10):3945-52.
4. Baldi PC; Miguel SE; Fossati CA; Wallach JC. Serological follow-up of human brucellosis by measuring IgM antibodies to lipopolysaccharide and cytoplasmic proteins of Brucella species. Clin Infect Dis 1996;22(3):446-55.
5. Casao MA; Leiva J; Diaz R; Gamazo C. Anti-phosphatidylcholine antibodies in patients with brucellosis. J Med Microbiol 1998; 47(1):49-54.

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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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	For the reagent blank, dispense 100ul sample diluent in 1A well position.
3	Add **100 ul Calibrators, Controls & diluted Sample** in appropriate wells.
4	Seal the plate and Incubate at Room Temperature for 20 minutes.
5	Aspirate and wash plate 3 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.
6	Add **100 ul** of **Enzyme Conjugate** to each well.
7	Incubate at Room Temperature for 20 minutes.
8	Repeat the Wash Step as mentioned in step 5.
9	Add **100 ul of TMB Substrate** into each well.
10	Incubate at room temperature for 10 minutes.
11	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 (96 wells)
POS CNTRL	Positive Control
NEG CNTRL	Negative Control
CAL	Calibrator
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