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DIAGNOSTIC AUTOMATION, INC.

21250 Califa Street, Suite 102 and 116,Woodland Hills, CA 91367
Tel: (818) 591-3030 Fax: (818) 591-8383
onestep@rapidtest.com
technicalsupport@rapidtest.com
www.rapidtest.com

IVD	See external label	2°C-8°C	96 tests	REF 1501-8

Brucella IgG ELISA

REF 1501-8

Test	Brucella IgG ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	Indirect ELISA : Antigen Coated Plate
Cut-off level	10U/mL
Sample	50ul serum/plasma
Specificity	100%
Sensitivity	100%
Total Time	~ 110 min
Shelf Life	12 Months from the manufacturing date

***** *Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*

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Intended Use

The Diagnostic Automation, Inc. Brucella IgG Antibody ELISA Test Kit has been designed for the the detection and the quantitative determination of specific IgG antibodies against Brucella in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of Diagnostic Automation Inc.

Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

General Information

Brucellosis is an infectious disease caused by small ellipsoid, gram-negative bacteria. There are four different germs: Br. abortus, Br. melitensis, Br. suis and Br. canis. People are infected by contact with infected animals or by eating meat or drinking unpasteurized milk from infected animals. As a rule, infected humans are not contagious. Brucellosis is most frequent in young and middle-aged individuals. Endangered persons are butchers, farmers, owners of pets, veterinaries and tourists in Southern countries. The appearance of brucellosis shows a prevalence during winter and spring.

The incubation period is between one and three weeks, but may be as long as two months. Br. abortus and Br. melitensis may cause Bang's Disease, or in rare cases Malta Fever. The first appears occasionally with a low pathogenicity for man. Typical symptoms for Bang's Disease are periodically occurring fever, splenomegaly and swelling of lymph nodes. In some cases an inflammation of different joints and organs occurs. The Malta Fever is caused by the epidemic type of brucellosis, and infection almost always leads to a manifest illness. Some infections with Brucella can cause Brucella Hepatitis. It is possible that there is a link between an infection with Brucella and the outbreak of multiple sclerosis.

During an antibiotic therapy or a chronic infection, the detection of Brucella spec. in blood, urine, cerebrospinal fluid, sputum or other body fluids could be negative. Serological methods like agglutination, complement fixation reaction, Brucella Coombs test and ELISA are good alternatives. The monitoring of antibodies can serve as a usual indication of the status of infection. During the first days, IgM is the only immunoglobulin that appears. As the disease progresses, IgM recedes quantitatively and IgG becomes predominant. In chronic brucellosis, IgG may be produced for an extended period.

Principle of the Test

The Diagnostic Automation, Inc. Brucella IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Brucella antigen is bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Brucella antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

Limitations, Precautions and General Comments

- Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.

- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

Reagents Provided

Storage and Stability (refer to the expiry date on the outer box label)

Store kit components at 2-8°C. After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. The opened kit should be used within three months.

Components	Volume / Qty.
Brucella antigen coated microtiter strips	**12**
Calibrator A (Negative Control)	**2 mL**
Calibrator B (Cut-Off Standard)	**2 mL**
Calibrator C (Weak Positive Control)	**2 mL**
Calibrator D (Positive Control)	**2 mL**
Enzyme Conjugate	**15 mL**
Substrate	**15 mL**
Stop Solution	**15 mL**
Sample Diluent	**60 mL**
Washing Buffer (10×)	**60 mL**
Plastic foils	**2**
Plastic bag	**1**

1. Microtiter Strips
12 strips with 8 breakable wells each, coated with a Brucella antigen (Brucella abortus, strain W99. Ready-to-use.

2. Calibrator A (Negative Control)

2 mL, protein solution diluted with PBS, contains no IgG antibodies against Brucella. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

3. Calibrator B (Cut-Off Standard)
2 mL human serum diluted with PBS, contains a low concentration of IgG antibodies against Brucella. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

4. Calibrator C (Weak Positive Control)
2 mL, human serum diluted with PBS, contains a medium concentration of IgG antibodies against Brucella. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5. Calibrator D (Positive Control)
2 mL, human serum diluted with PBS, contains a high concentration of IgG antibodies against Brucella. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

6. Enzyme Conjugate
15 mL, anti-human-IgG-HRP (rabbit), in protein-containing buffer solution. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane and 5 mg/L Proclin™. Ready-to-use.

7. Substrate
15 mL, TMB (tetramethylbenzidine). Ready-to-use..

8. Stop Solution
15 mL, 0.5 M sulfuric acid. Ready-to-use.

9. Sample Diluent
60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.

10. Washing Buffer
60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

11. Plastic Foils
2 pieces to cover the microtiter strips during the incubation.

12. Plastic Bag
Resealable, for the dry storage of non-used strips.

Materials Required but not Provided

- 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- Microtiter Plate Reader (450 nm)
- Microtiter Plate Washer
- Reagent tubes for the serum dilution
- Deionized Water

Specimen Collection and Handling

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 7 days. For a longer storage they should be kept at -20°C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

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Assay Procedure

1. Preparation of Reagents

Washing Solution: dilute before use 1+9 with deionized water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- A standard curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

2. Assay Steps

2.1 Prepare a sufficient amount of microtiter wells for the standards, controls and samples in duplicate as well as for a substrate blank.

2.2 Pipet 100 µL each of the **diluted** (1:101) samples and the **ready-to-use** standards and controls respectively into the wells. Leave one well empty for the substrate blank.

2.3 Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.

2.4 Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.

2.5 Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.

2.6 Cover plate with the enclosed foil and incubate at room temperature for 30 minutes.

2.7 Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.

2.8 Pipet 100 µL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.

2.9 Cover plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g. drawer).

2.10 To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.

2.11 After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

Evaluation

Example

	OD Value	corrected OD
Substrate Blank	**0.020**	
Negative Control	**0.077**	**0.057**
Cut-Off Standard	**0.537**	**0.517**
Weak Positive Control	**1.069**	**1.049**
Positive Control	**2.041**	**2.021**

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently **no reference values** which have to be found in other laboratories in the same way.

1. Qualitative Evaluation

The calculated absorptions for the patient sera, as mentioned above, are compared with the value for the cut-off standard. If the value of the sample is higher, there is a positive result. For a value below the cut-off standard, there is a negative result. It seems reasonable to define a range of +/-20 % around the value of the cut-off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same patient, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run.

The positive control must show at least the double absorption compared with the cut-off standard.

2. Quantitative Evaluation

The ready-to-use standards and controls of the Brucella IgG antibody kit are defined and expressed in arbitrary units (U/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible. The values for controls and standards in units are printed on the labels of the vials.

For a quantitative evaluation the absorptions of the standards and controls are graphically drawn *point-to-point* against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs. As curve fit *point-to-point* has to be chosen.

Calibrator B with its concentration of 10 U/mL serves as cut-off standard. Analogous to the qualitative evaluation a range of +/-20% around the cut-off is defined as a grey zone. Thus results between 8 and 12 U/mL are reported as borderline.

Performance Characteristics

Brucella ELISA	IgG	IgA	IgM
Intra-Assay-Precision	**8.7 %**	**9.3 %**	**9.2 %**
Inter-Assay-Precision	**7.9 %**	**8.4 %**	**5.0 %**
Inter-Lot-Precision	**4.1 – 8.8 %**	**4.4 – 10.5 %**	**1.2 – 7.1 %**
Analytical Sensitivity	**1.11 U/mL**	**1.14 U/mL**	**1.03 U/mL**
Recovery	**82 – 96 %**	**92 – 114 %**	**85 – 110 %**
Linearity	**80 – 115 %**	**69 – 110 %**	**75 – 110 %**
Cross-Reactivity	**No cross-reactivity to Bordetella pertussis**		
Interferences	**No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL**		
Clinical Specificity	**100 %**	**100 %**	**100 %**
Clinical Sensitivity	**100 %**	**100 %**	**100 %**

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EC REP	**CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands.** www.cepartner4u.eu

DIAGNOSTIC AUTOMATION, INC.

21250 Califa Street, Suite 102 and 116, Woodland Hills, California 91367 USA

Tel: (818) 591-3030 Fax: (818) 591-8383

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