INTENDED USE

IgG may be produced for an extended period. As the disease progresses, IgM recedes quantitatively. During the first days, IgM is the only immunoglobulin that can serve as a usual indication of the status of infection. Test and ELISA are good alternatives. The monitoring of antibodies is directly proportional to the intensity of the color. Tests have additionally to be taken into account.

GENERAL DESCRIPTION

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 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.
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.

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

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

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

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.

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

Plastic Bag: Reusable, for the dry storage of non-used strips.

MATERIALS REQUIRED BUT NOT PROVIDED

1. 5 μL-, 100 μL- and 500 μL micro- and multichannel pipets
2. Microtiter Plate Reader (450 nm)
3. Microtiter Plate Washes
4. Reagent tubes for the serum dilution
5. Bidistilled 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 48 hours, 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).

ASSAY PROCEDURE

1. Reagent And Sample Preparation

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

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

2. Assay Steps

1) Prepare a sufficient amount of microtiter wells for the standards, controls and samples in duplicate as well as for a substrate blank.
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.
3) Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.
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.
5) Pipet 100 μL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
6) Cover plate with the enclosed foil and incubate at room temperature for 30 minutes.
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.
8) Pipet 100 μL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.
9) Cover plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g. drawer).
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.
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

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10%.

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 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 calibrators are graphically drawn against their concentrations. From the resulting calibration 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.

REFERENCES


ASSAY CHARACTERISTICS

<table>
<thead>
<tr>
<th>Brucella ELISA</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-Assay-Precision</td>
<td>8.7 %</td>
<td>9.3 %</td>
<td>9.2 %</td>
</tr>
<tr>
<td>Inter-Assay-Precision</td>
<td>7.9 %</td>
<td>8.4 %</td>
<td>5.0 %</td>
</tr>
<tr>
<td>Inter-Lot-Precision</td>
<td>4.1 – 8.8 %</td>
<td>4.4 – 10.5 %</td>
<td>1.2 – 7.1 %</td>
</tr>
<tr>
<td>Analytical Sensitivity</td>
<td>1.11 U/mL</td>
<td>1.14 U/mL</td>
<td>1.03 U/mL</td>
</tr>
<tr>
<td>Recovery</td>
<td>82 – 96 %</td>
<td>92 – 114 %</td>
<td>85 – 110 %</td>
</tr>
<tr>
<td>Linearity</td>
<td>80 – 115 %</td>
<td>69 – 110 %</td>
<td>75 – 110 %</td>
</tr>
<tr>
<td>Clinical Specificity</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Clinical Sensitivity</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
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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.