

Diagnostic Tests in Human Brucellosis

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Context: Brucellosis represents a zoonotic bacterial disease, caused by a gram negative bacterium called *Brucella*. Between the diverse species of this bacteria, *B. melitensis*, *B. abortus*, *B. suis* and *B. canis* consist the main causes of the disease in humans. More than half a million new cases of Brucellosis are reported annually. Consequently, brucellosis is a remarkable threat for the health of society. Because of the multiple nonspecific clinical signs of this infection, such as fever (60% of cases), night sweating, insomnia and anorexia, which are similar to other diseases, the detection of brucellosis is time-consuming and needs more scrutiny.

Evidence Acquisition: Blood culture is considered the gold standard for the detection of brucellosis and the sensitivity of this test in the acute form is high. However, for the chronic type of disease, it is remarkably low, in addition, in some cases, it needs long reaction times. Nevertheless, today, some kinds of tests like automatic culturing system and serological methods, such as Rose Bengal (RB) test, serum agglutination test (SAT), 2-mercaptoethanol (2ME) and coombs, which are operated based on agglutination, are useful for the problems mentioned earlier.

Conclusion: Although serological methods are common for the diagnosis of brucellosis, false results are observable for several methods, such as the SAT method. Tests like the enzyme-linked immunosorbent assay (ELISA), for the screening of specific traits, although confirmed, have their advantages and defects. The lateral flow assay (LFA) shows promising evidence to be effective in the diagnosis of brucellosis. The polymerase chain reaction (PCR) is more prevalent than other common tests, according to sensitivity and fast answering potency in case of molecular diagnosis. Also, PCR is proper for patients' follow-up during the period of treatment and crimation of relapse by this method is easier compared to others.

Keywords: Brucellosis; *Brucella*; Diagnosis; Polymerase Chain Reaction; Lateral Flow Assay

1. Context

Brucellosis is one of the most important zoonotic diseases around the world. The epidemiological region of this disease includes the Mediterranean area, Arab peninsula, Indian subcontinent and some parts of South and Central America (1). This disease was discovered for the first time by Dr. David Bruce. In 1886, he isolated a coccobacillus, called *Micrococcus melitensis*, from the spleen of a person who had died of "Malta fever" disease (2). In 1897, *Brucella abortus* was isolated by Bang (3), and in 1914 *B. suis* was discovered by Traum (4). In 1968, *B. canis* was first recognized by Carmicheal and Bruner. Meanwhile, each of these four species had been recognized as a pathogen in humans (5). However, now, *B. melitensis* is the main cause of brucellosis infection in humans (6). Other species of *Brucella* account for *B. ovis*, *B. neotomae*, *B. microti*, *B. cetaceae* and *B. inopinata* (7-9).

Brucella bacterium is a gram-negative, intracellular, aerobic and non-capsulated coccobacillus, (5, 7). The lipopolysaccharide (LPS) of the bacteria is considered as critical antigen for survival in the host's body, so that

this antigen engages in the prevention of infected cells apoptosis (10, 11). Cytoplasmic antigens, periplasmic and outer membrane structural proteins like (OMPs) are important elements which are recognized by the host's immune system (12, 13).

The consumption of unpasteurized dairy products, like raw milk (cow, sheep, goat), soft cheese and butter, represents the most usual way of transmission for this disease (6, 14). Inhalation and transmission of the microorganism by laboratory staff has also been reported (15). Moreover, *brucella* bacteria might be transmitted through skin superficial lesions (16). Every system and every organ can be affected by this infection (17). Hematologic, respiratory, gastrointestinal, osteoarticular, neurologic and cardiovascular disorders may be observed in these patients (18). It is necessary to mention that between these disorders, osteoarticular involvement is the most frequent (19, 20). Heart disorders or endocarditis account for the highest mortality rates in this disease (21). The incubation period of this infection

is variable (2-3 weeks on average). The main complaints of infected patients consist of fever, night sweats, headache, backache and anorexia (22, 23). The cardinal symptom of brucellosis is fever with unfamiliar origin, so that it can be misdiagnosed with similar pathologies such as malaria, tuberculosis, rheumatic fever, malignancy and leishmaniasis (22-26).

A series of findings like medical history, clinical findings, routine biochemical tests and some tests such as radiographs, magnetic resonance imaging (MRI), are useful in the diagnosis of this infection. In endemic areas, epidemiological information related to brucellosis is also highly important. It is important to note that the usage of cultures, serological and molecular tests for diagnosis is crucial, as this article will further explain.

2. Evidence Acquisition

2.1. Culture

One of the gold standards for brucellosis diagnosis is blood culture. The Biphasic Ruiz-Castaneda system is a conventional way for the isolation of *brucella* bacteria from infected patients (27). This method has a long incubation period of about 6 weeks, but the sensitivity of this method for the acute form had been reported as lower than 90% and in the chronic form, to be as low as 20% (28, 29). Nowadays, numerous methods, like the lysis-centrifugation method, have replaced previous usual diagnostic methods (30). In this method, the average detection time had been decreased (31). Also, the sensitivity of this technique for the acute form is of about 90% and for the chronic form, less than 70% (30, 32). The previous automated culture systems were principally represented by the Bactec methods, such as the case of Bactec 9204 and Bact/Alert. It should be mentioned that persons who are working with this technique to avoid microorganism contamination through contaminated dust, must keep in mind the sanitary notes (33). The bone marrow (BM) culture method is another diagnostic method, in which the detection time had been significantly decreased and the sensitivity of this method is higher than 90%. Although the biopsy of BM is very painful, this diagnostic method is valid and valuable for patients who have fever with unfamiliar origin that accompany with negative serological tests and suspicious signs of brucellosis (34-37). In other cases, there is a local involvement of brucellosis disease, and it is necessary that the sample for culture to be from the organ involved, like a lymph node or liver (38, 39).

The laboratory diagnosis of brucellosis is mainly based on the presence of serum antibodies (40). Because of the low sensitivity and risks of the culture method, the presence of infection is mostly evidenced by serological methods that can show increments of the titer of antibodies (Ab). Different serological tests based on agglutination have been designed and implemented. These tests consist of the Rose Bengal test (RB), serum agglutination

test (SAT), 2-mercaptoethanol test (2ME) and the Coombs test (41).

2.2. Rose Bengal Test

The RB is usually used for brucellosis screening, yet experts propose that the result of this test must be approved by some special tests (42, 43). This test is easily performed, is fast and has a high sensitivity, within 99% the diagnosis of acute patients. Specificity in RB is low, in the chronic form of infection, the probability of false-negative result for this test is high (44).

The Rose Bengal Plate (RBP) test in high risk rural areas, in that doing of serum agglutination test (SAT) is not possible, has been introduced as a screening test and it is highly valuable. However, this test result must also be approved by another tests (7).

2.3. SAT

The SAT, introduced by Wright et al. used a strain of *brucella abortus* 119 with antibodies against *B. abortus*, *B. suis* and *B. melitensis*, which caused the agglutination reaction. However, SAT is not useful for *B. canis* detection. The SAT can estimate the total quantity of IgM and IgG (45). These antibodies indeed act against the smooth Lipopolysaccharide (LPS). However, sometimes *brucella* LPS has cross-reactions with some bacteria like *Salmonella* O30 and *Escherichia hermanni*, in which the results are false-positive (46).

In developing countries, a SAT titer $\geq 1:320$ with a 2-mercaptoethanol (2ME) titer $\geq 1:80$, coupled with clinical symptoms, are the most important methods for brucellosis diagnosis. However, in endemic areas with more patients suffering from acute bacteremia, the SAT titer threshold is of at least 1:320 (47). As SAT in the chronic form has sometimes false-negative results, several studies have showed that in the first few days of the disease, even bacteremic patients had an SAT titer $\leq 1:160$ (48). The IgG antibody, which is diagnosed by SAT, can persist 2 years after the successful treatment of this disease and induces a positive SAT response (49). The 2ME inactivates the agglutination trait of IgM and also determines the quantity of specific IgG in the acute form of disease.

2.4. Coombs Test

Coombs test is useful for antibodies detection, like the blocking of IgG in patients who are suffering from the chronic form of disease (50, 51). Existence of a block on an antibody or Prozone phenomenon cause false-negative SAT results, and therefore the usage of the Coombs test is a ideal method to overcome this problem (52, 53). In brucellosis diagnosis, the SAT test is the most trustworthy test, yet in some patients who have obvious clinical symptoms and negative SAT results, it is better to use the Coombs and enzyme-linked immunosorbent assay (ELISA) methods (54). The Coombs test is the most sensitive test for the confirmation of brucellosis relapsing (45).

2.5. ELISA

The ELISA is a fast test and has a high sensitivity and specificity about 80% the diagnosis of IgM, IgG and IgA antibodies related to *brucella* in blood (55-57). This test can diagnose an incomplete antibody and this antibody is generally observable in chronic patients with brucellosis, therefore recommending this test for such patients (58)

Some tests, such as the SAT and RB, might misdiagnose positive cases in chronic and complicated patients, therefore making the ELISA more desirable (59). It is also useful for mass screening of brucellosis as well as RBT, because the setting up process of this test is very difficult (60). The use of ELISA for the diagnosis of neurologic brucellosis has a high sensitivity (45). However, even if several studies have shown that the sensitivity of ELISA is lower than SAT (61, 62), the ELISA test is an acceptable offer in comparison with the SAT (63). Immunochromatography lateral flow assay (LFA) test is a simplified form of ELISA. This test can be used for the diagnosis in all levels of disease (from acute to chronic). Some significant characteristics of the LFA test are the fast accomplishment and easy interpretation and also a sensitivity and specificity > %90 (42).

2.6. PCR

The polymerase chain reaction (PCR) test is a molecular detection test, used for the detection of the brucellosis microorganism in humans and animals. However, there are limited studies concerning the usage of this test in animals (64, 65). This technique is faster than conventional methods, while the usefulness and sensitivity of this method had been also proved (66). The PCR test is used for the detection of DNA microorganisms (67). The DNA of the *brucella* bacteria is recognizable in serum, blood, pus and tissue, yet the usage of a blood sample for the detection of brucellosis in humans by PCR test is more usual. However, usage of serum for detection is more popular and has priority compared to blood (68, 69). For the omission of prohibitory factors, which are effective in PCR results from blood samples, such as leukocyte DNA with high density (70, 71), the utilization of a more useful method for DNA extraction is recommended. The PCR system uses primer pairs for the detection of *brucella*. These primers used for sequence encoding are 16 Sr RNA (72), 16 S23 r DNA (73), BCP 31 (B4/B5) (74) and outer membrane protein like 31 (OMP31) (75). This test is highly useful in the detection of early relapses (17). However, the detection of relapse by conventional method is very difficult. In some studies, patients surveyed by PCR, after accomplishment of their treatment, have evidenced the bacteria in blood for a partly longer term brings into discussion the possibility of relapse (76). Relapse usually occurs within 6 months after cessation of treatment (47). Consequently, patients must pursue a treatment plan for 12 months. Indeed, the relapse is regarded as major variable in the therapeutic regimen assessment for brucello-

sis patients (77). Also, PCR can be used for monitoring the efficacy of treatment. However, the PCR test needs several instruments for accomplishment and the probability of contamination is high in this method. Therefore, the real time PCR method had expanded and it has a faster and easier application in clinic, with a lower contamination probability than the PCR (78, 79).

4. Conclusions

The existence of some tests that are fast, cheap, and have high sensitivity and specificity for brucellosis detection are necessary, so finding of diagnostic methods are one of the major issues which needs further research.

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