MODERN LABORATORY DIAGNOSTIC METHODS FOR CHRONIC BRUCELLOSIS: COMPARISON OF PCR, ELISA, AND ROSE BENGAL TEST

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Abstract. Chronic brucellosis poses significant diagnostic challenges due to its nonspecific clinical manifestations and prolonged course. Laboratory confirmation remains the cornerstone of diagnosis. Among the available techniques, serological assays such as the Rose Bengal (RB) test and ELISA, and molecular methods like polymerase chain reaction (PCR), play crucial roles. This review analyzes the comparative effectiveness of these methods in terms of sensitivity, specificity, rapidity, and applicability in different clinical and epidemiological contexts.

Keywords: chronic brucellosis, diagnosis, PCR, ELISA, Rose Bengal test, sensitivity, serology

Introduction. Brucellosis is a zoonotic disease caused by Brucella spp., with B. Melitensis being the most virulent and frequently implicated in human infections [1]. Chronic brucellosis develops when infection persists beyond 12 months, often presenting with nonspecific symptoms such as fatigue, arthralgia, and low-grade fever [2]. As clinical features alone are insufficient, laboratory confirmation is essential. The three most widely used methods include the Rose Bengal test (RB), enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR).

Rose Bengal Test (RB). The RB test is a rapid slide agglutination assay that detects antibodies against Brucella antigens. It is inexpensive, simple to perform, and widely used as a screening test in endemic areas.

Advantages: High sensitivity in acute brucellosis (≥90%), Inexpensive and suitable for field conditions, Fast results (within 5–10 minutes)

Limitations: Low sensitivity in chronic infections due to declining IgM titers [3]

Cross-reactivity with other Gram-negative bacteria, Subjective interpretation of agglutination

As noted by Memish Z. A. Et al., the RB test shows decreasing sensitivity with disease chronicity, missing up to 40% of chronic cases [3].

Enzyme-Linked Immunosorbent Assay (ELISA). ELISA enables the quantitative detection of IgM, IgG, and IgA antibodies against Brucella. It offers better sensitivity and specificity compared to the RB test, especially in chronic and relapsing brucellosis [4].

Advantages: Differentiation of acute (IgM) and chronic (IgG) phases, high throughput and reproducibility, suitable for follow-up of therapy response

Limitations: False positives due to persistence of IgG after recovery, Requires laboratory infrastructure and trained personnel.

According to Mantur B. G. Et al., ELISA detects chronic cases with 95% sensitivity and 100% specificity, making it superior to RB in long-standing infections [4].

Polymerase Chain Reaction (PCR). PCR directly detects Brucella DNA in blood or tissue, making it especially useful in cases with low or absent antibody titers. Targets include the bcsp31, IS711, and omp2 genes [5].

Advantages:Detection of low bacterial loads, early diagnosis before seroconversion, Useful in immunocompromised patients or atypical presentations

Limitations: Risk of contamination and false positives, expensive and requires technical expertise, DNA persistence may lead to false positives after treatment

Comparative analysis of diagnostic methods in chronic brucellosis. The diagnosis of chronic brucellosis remains a clinical challenge due to the nonspecific nature of symptoms, intermittent bacteremia, and often low immune response. This necessitates the integration of multiple laboratory diagnostic modalities for accurate identification. In this chapter, we provide an in-depth comparison of the three key diagnostic tools—Rose Bengal test (RB), enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR)—based on sensitivity, specificity, applicability in chronic cases, cost, and turnaround time.

Sensitivity and Specificity. Sensitivity and specificity are essential parameters for evaluating diagnostic test performance. Studies have shown that RB test, while useful in acute infection, loses sensitivity in chronic stages. According to Memish et al., sensitivity of RB drops below 60% in chronic infections due to the decline in IgM antibodies over time [3].

In contrast, ELISA demonstrates high sensitivity (95%) and specificity (up to 100%) in chronic cases due to its ability to detect long-lasting IgG and

IgA antibodies**.** Mantur et al. emphasize that ELISA is significantly more reliable for chronic brucellosis than agglutination-based tests [4].

PCR, detecting bacterial DNA directly, maintains a high sensitivity (80–100%) and specificity (95–100%), particularly when blood or tissue samples are used. It is especially valuable in cases where antibody levels are low or serological tests are negative, as confirmed by Al Dahouk et al. [5].

Diagnostic timing and turnaround. The RB test offers the quickest results**,** within 10–15 minutes, making it ideal for rapid screening in endemic or resource-limited areas [3]. ELISA and PCR are more time-consuming (2–6 hours) but provide quantitative and specific results**,** essential for clinical decision-making.

Usefulness in chronic brucellosis. Chronic brucellosis is characterized by fluctuating or absent antibodies and low bacteremia. Thus, RB is poorly **suited** for diagnosis in such cases. ELISA, by differentiating antibody classes (IgG vs IgM), provides a better clinical correlation, helping to distinguish between active and past infections [4]. PCR surpasses serological methods in cases of latent, intracellular infection, relapse, or immunosuppression, where antibodies may be insufficiently produced or no longer detectable. Navarro et al. stress its value in monitoring treatment response and relapse diagnosis [6].

Practicality and Resource Requirements. The RB test is inexpensive, portable, and easy to perform, making it ideal for screening large populations**.** However, its low specificity and limited use in chronic disease reduce its standalone diagnostic value [3]. ELISA requires laboratory infrastructure, reagents, and trained staff**,** but its ability to detect multiple antibody classes improves its reliability [4]. PCR is the most resource-intensive, demanding high-level laboratory conditions to avoid contamination and ensure reproducibility, as noted by Al Dahouk et al. [5].

Conclusion. The RB test remains a useful initial screening tool, particularly in resource-limited settings. However, its limited sensitivity in chronic brucellosis necessitates the use of ELISA and PCR for confirmatory testing. ELISA offers reliable antibody profiling, while PCR enables direct detection of Brucella DNA, especially when serology is inconclusive. An integrated diagnostic approach combining serological and molecular assays ensures the highest diagnostic accuracy, especially in chronic or atypical cases.

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