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Review Article

Microbiology Section

Advances in *Brucella* Detection: A Comprehensive Review of Traditional and Emerging Techniques

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ABSTRACT

Brucellosis remains a major zoonotic infection across the world. *Brucella melitensis*, *B. abortus*, and *B. suis* are considered to be most frequently attributed to human infection and transmission may occur either as a direct contact with infected animals or ingestion of unpasteurised animal products. The diagnostic issues are complex in nature due to the non specific clinical presentation of the condition and infrastructure constraints of the traditional laboratory techniques, particularly in resource-limited settings. This review compares both conventional and novel diagnostics for the detection of *Brucella*, and presents the advantages and limitations of each. The older methods, including Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (SAT), Enzyme-Linked Immunosorbent Assay (ELISA), and bacterial culture, remain popular, but lack sensitivity, have variable specificity, and are costly in terms of resources. In comparison, molecular methods such as Polymerase Chain Reaction (PCR), quantitative PCR (qPCR), nested PCR and droplet digital PCR (ddPCR) are more sensitive and have a faster turnaround time, but require an advanced laboratory infrastructure. Improvements in the development of diagnostics like CRISPR-Cas-based assays and lateral/vertical flow immunoassays suggest great potential in the easier deployment in the field. The review hence underlines the dire need of low-cost, high speed and highly accurate diagnostic tools that can discriminate at the species level. The priorities of future research are the incorporation of the one health point of view, the systematic standardisation and multicentre validation of new diagnostic platforms.

Keywords: Brucellosis, Diagnostic techniques, Immunoassay, Molecular detection, Point-of-care systems

INTRODUCTION

Brucellosis is a bacterial zoonotic disease that imposes a considerable disease burden worldwide, particularly in the Middle East, Africa, and Asia [1]. The disease develops by infection with intracellular gram-negative coccobacilli belonging to the family Brucellaceae. The predominant instances of human diseases are linked to infections caused by *Brucella abortus* or *B. melitensis* [2]. The disease primarily spreads to humans through contact with diseased animals and the consumption of contaminated meat or unprocessed dairy products [3]. As presently known, six *Brucella* species have been identified to possess zoonotic potential [4]. Most common pathogens associated with human brucellosis are B. *melitensis*, *B. abortus*, and *B. suis.* Annually, around 500,000 new human cases occur globally, mostly in low- and middle-income countries [5]. The extensive variety of hosts, diverse transmission pathways, effects on animal and human welfare, and the global distribution of affected countries indicate why One Health in brucellosis has been the subject of at least ten publications, either broadly or in particular environments [6]. The prevalence of *Brucella* spp. infection in livestock is typically greatest in arid and semiarid regions, especially where cattle, small ruminants, or camels are raised in substantial herds or flocks under elaborate management practices [7].

Human brucellosis symptoms manifest as an acute febrile sickness that may evolve into a chronic condition marked by flu-like symptoms and musculoskeletal pain [8]. The typical manifestation of human brucellosis is a non specific febrile disease, typically characterised by clinical symptoms such as headache, malaise, fatigue, and discomfort in the back and joints [9]. The disease can develop chronic and may impact any organ system, leading to serious conditions such as osteomyelitis [10], epididymo-orchitis [11], neurological disorder [12], and cardiovascular complications [13]. Brucellar spondylitis and spondylodiscitis predominantly impact the lumbar region (60%), with less frequency in the thoracic region (19%) and infrequently the cervical portions (12%). Spinal epidural abscess is a more severe clinical manifestation that arises related to spondylodiscitis [14]. The clinical identification and treatment of human brucellosis continues to cause significant challenges in numerous endemic countries. Brucellosis cannot be diagnosed only based on clinical signs and symptoms; a conclusive diagnosis requires the identification of *Brucella* spp. using culture or molecular methods, or the demonstration of elevated levels in paired acute and convalescent serological testing [15]. The poor accuracy of tests leads to misdiagnosis and inappropriate management. The diagnosis and treatment of human brucellosis is a major challenge in those countries where this disease is endemic. Healthcare facilities and laboratories in low-resource environments endemic to brucellosis encounter numerous obstacles in diagnosing human brucellosis. The suggested diagnostic techniques (e.g., culture and serological analysis of paired sera utilising the SAT) are technically challenging, exhibit rather prolonged turnaround times, and are costly and frequently inaccessible in numerous endemic environments. Numerous commercially accessible plate agglutination tests, referred to as fast or febrile antigen *Brucella* agglutination tests, are extensively utilised in healthcare facilities across East Africa, perhaps owing to their perceived cost-effectiveness and ease [16]. Consequently, further evidence is required to correctly evaluate the performance attributes of the variety of kits in utilisation and their cost-effectiveness [17]. However, molecular approaches focused on nucleic acid detection and amplification provide a more rapid turnaround time and, in many cases, greater sensitivity than bacterial culture methods [18]. Conventional serological tests identify the patient's antibody response to the bacterial pathogen by agglutination, complement activation, immunoprecipitation processes, or primary binding assays such as ELISA or lateral flow immunochromatography assays [19]. All these assays identify antibodies targeting the lipopolysaccharide antigen located on the

outer membrane of smooth *Brucella* species, including *B. melitensis*, *B. abortus*, and *B. suis* [20].

The Febrile Brucellin Antigen Test (FBAT) is a modification of the rapid slide FBAT, frequently employed in East African nations due to its low price and simplicity of use [21]. The Rose Bengal Test (RBT) is a quick slide agglutination assay at pH 3.7. It has demonstrated great diagnostic sensitivity and can identify *Brucella*-specific antigenic stimuli in endemic environments. Furthermore, it is comparatively cost-effective and necessitates the same fundamental laboratory apparatus and proficiency as the FBAT, rendering it ideal for small laboratories with limited funding [22]. False positives may arise via cross-reactivity with non target diseases or from the detection of antibodies resulting from prior exposure, rather than indicating a present sickness, creating a considerable issue in brucellosis-endemic regions [23].

Nucleic Acid Amplification Tests (NAATS) are regarded as safer, faster, and more effective alternatives for diagnosing brucellosis, encompassing traditional, real-time, and multiplex PCR techniques [24]. Regarding NAATs, a significant challenge is to address the issue of false-negative results caused by inhibitors present in clinical samples and the cumbersome nature of analytical sensitivity [25]. Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) has been regarded as an exemplary solution to address the aforementioned challenges in certain laboratories. In comparison to RT-qPCR, dd-PCR is a revolutionary technique. The nucleic acid amplification approach facilitates absolute quantification of target nucleic acids, exhibits enhanced tolerance to inhibitors, and eliminates the necessity for a reference curve, making it suited for various disease stages and low-concentration samples [26]. Molecular diagnosis can be laborious and necessitates costly chemicals, apparatus, and skilled laboratory staff. Lateral Flow Immunoassay (LFIA) is extensively reported for the quick diagnosis of brucellosis due to its portability, ease of operation, and its lack of need for costly apparatus and specialised laboratory workers for result interpretation. A very sensitive dual-colour rapid RBT utilising gold nanoparticles was developed for the quick serodiagnosis of brucellosis [27]. Vertical Flow Immunoassay (VFIA) differs from RBT (LFIA), with its operational basis grounded in vertical filtering. In VFIA, the nitrocellulose (NC) membrane serves as the solid support. The nanoparticle conjugate serves as the detection probe, supported by the antigen or antibody, while the particular immunological affinity reaction is conducted utilising the filterability of the NC membrane. In comparison to LFIA, VFIA offers the benefits of reduced detection time and the absence of a hook effect [28]. Recent developments in genetic engineering to replace S-LPS have led to the creation of innovative ELISA assays utilising non LPS *Brucella* immunodominant antigens, either as pure natural proteins or as multi-epitope recombinant proteins [29].

This review aim to provide a detailed comparison of the traditional as well as modern diagnostic methods used in the detection of human brucellosis. It assesses the effectiveness of such methods in terms of sensitivity, specificity, and reliability as well as exploring its viability in the context of normal clinical practice. A special focus is given to the difficulty in resource-limited and endemic environments, in which proper diagnosis is often the biggest obstacle.

Traditional Brucella Detection Methods

Rose Bengal Plate Test (RBPT): RBPT is a popular screening technique used in the diagnosis of Brucellosis, as it is simple, quick and cheap. Its principle consists in agglutination of stained *Brucella* antigens with serum of a patient and is especially well adapted to field practice. The sensitivity (96.0%) and specificity (90.7%) are high, which proves the reliability of the test as a first-line procedure with the help of empirical data. Similar results were recorded by Almashhadany DA et al., who indicated 100% sensitivity and 96.9% specificity in human brucellosis cases [28]. In contrast, Barkay O et al., warn that RBPT may produce false negatives of acute infections, and Legesse A et al., recorded false positives in small ruminants and false negatives in cattle [29,30]. Nevertheless, RBPT is a valuable diagnostic tool in human and veterinary practice and particularly in resource-constrained settings despite these limitations. Blood samples were tested for *Brucella* spp. antibodies using RBT and blood culture followed by species identification; About 10 mL of venous blood was collected from each participant, about, 5-7 mL were inoculated into an aerobic blood culture bottle, and then 3 mL were collected into a plain tube for RBT [28].

Standard Tube Agglutination Test (SAT): SAT is a common quantitative serological test that is used in diagnosis of *Brucella* infection. The test methods give a practical way of diagnosing *Brucella* by quantifying the production of visible agglutinates in a tube following exposure of patient sera to *Brucella* antigens. The sensitivity was 89.2, and specificity was 95.6, which proves its diagnostic value (Freire ML et al., However, there is no distinction between active and past infections in SAT [31]. Wang H et al., mentioned that in chronic cases of brucellosis, SAT-detected antibody titers were persistently present after treatment, thus emphasising its minor application in confirming a treatment [32]. Also, SAT is ceased to be commercially available in many areas, which is also noticed by Loubet P et al., although SAT can be used as part of a complex diagnostic approach, the difficulty of interpretation and diminished usefulness in chronic patients limits its usefulness alone [33].

Coombs test (Antiglobulin Test): The antiglobulin test (sometimes called Coombs test) detects non agglutinating or blocking antibodies that cannot be detected by SAT or RBPT. Its special usefulness is in the detection of low-titer antibodies particularly in chronic or complicated brucellosis. Barkay O et al., record a specificity of 98.8% and a sensitivity of 89.4% [29]. Altunçekiç Yildirim A et al., also proved its usefulness when they incorporate Coombs testing into their diagnostic procedure, [34]. However, the process is not so common in the field due to its technical requirement and inaccessibility to rural areas. Nevertheless, the Coombs testing is also a necessary adjunct when the outcome of RBPT or SAT is rather inconclusive.

Complement Fixation Test (CFT): Since its invention the Complement Fixation Test (CFT) has been used as a confirmatory test and this is particularly so in the verification of *Brucella* specimens in international animal trades. A study conducted by Legesse A et al., showed that CFT has the lowest sensitivity compared to the rest of the conventional assays but has a 100% specificity, which highlights its high specificity and low sensitivity, especially when the infection is still early or latent [30]. The fact that the assay needs functional complement and anti-complementary substances also makes interpretation more difficult. As a result, CFT is rarely used nowadays, mainly because of the difficulties of practical application and the existence of more accurate surrogates, like ELISA and PCR, which have to a large degree replaced it.

Enzyme-Linked Immunosorbent Assay (ELISA): ELISA is one of the most commonly used serological techniques in the diagnosis of *Brucella* and allows has high sensitivity and specificity of IgM and IgG antibodies detection. In one study by Freire ML et al., ELISA showed a sensitivity of 96.8% and specificity of 98.6% which was higher than most of the conventional agglutination tests [31]. In addition, the assay is also very good at differentiating acute (IgM) and chronic (IgG) infections. The study by Xu N et al., revealed that the anti-*Brucella* IgG is the most reliable biomarker of complicated brucellosis [35], and the study by Dong SB, has found that the combination of ELISA and the Fluorescence Polarisation Assay (FPA) has almost perfect diagnostic accuracy [3]. However, Liu H et al., pointed out the need for wider validation in cohorts of a larger

sample size and the presence of false-positive reactions in cross-reactive bacterial infections [36]. Blood samples were collected and then centrifuged to separate the serum from the blood. Serum was tested for the presence of antibodies to *Brucella* spp. using a Febrile *Brucella* Agglutination Test (FBAT) (Febrile Serodiagnostics tests) and for molecular techniques [37].

Bacterial culture: The culture of bacteria remains the official standard of confirmatory diagnosis of *Brucella*, allowing species determination and antimicrobial susceptibility testing. As Feng Q et al., found 8.3% cases of aerobic blood culture isolates *Brucella*, thus showing its poor sensitivity [38]. In contrast, Barkay O et al., found sensitivity of 34.8% and specificity of 100% [29]. The gold standard for diagnosing *Brucella* in a lab setting is culture; specimen types include tissue samples, blood, bone marrow, cerebrospinal fluid, pus, and synovial fluid [38]. Even though culture ensures the accuracy of diagnosis, its protracted turnaround time, the requirement of biosafety level 3 containment and lack of applicability in endemic areas combine to reduce the clinical utility of culture. Konya P et al., stated that it is useful in research but emphasise that it has little practical applicability in daily diagnostics [39].

Molecular Detection Techniques

Conventional Polymerase Chain Reaction (PCR): The common use of PCR in detecting *Brucella* Deoxyribonucleic acid (DNA) has been through the amplification of specific target genes. As Khurana SK et al., emphasised, PCR is a significant addition to the serological test since it allows identifying *B. abortus* with greater accuracy [40]. However, the method cannot distinguish between the field and vaccine strains, thus limiting its epidemiologic application. Dadar M et al., have found similar results, as the most common methodology of diagnosis in Iranian livestock research was PCR, but there were significant disparities in geographical coverage and even standardisation of tests [41]. Qureshi KA et al., [42] also indicated that despite the contribution of PCR to the knowledge of pathogenesis, it has the disadvantages of false-positive rates and failure to distinguish between active and past infections. Lastly, Freire ML et al., assessed the sensitivity of PCR as moderate (79.6%) and the specificity as high (96.0%), and these values were affected by temporal, target-specific DNA variation, and prior antibiotic treatment [31]. The molecular diagnostic PCR targeting the *Brucella* genus-specific bcsp-31 gene in patients having febrile illness [43]. The bcsp31 gene synthesies an immunogenic membrane protein and thus serves as the most widely employed target for the molecular diagnosis of *Brucella* infection [44].

Nested PCR: The nested PCR is known as the PCR amplification technique used to increase sensitivity of the assay to such extent that even when bacterial load is low, *Brucella* DNA can be detected using two sets of primers applied. According to Rahbarnia L et al., blood-based nested PCR had 68.18% sensitivity and 100% specificity as compared to serum PCR, SAT, and culture [45]. The technique was especially beneficial in chronic cases of brucellosis where other methods were of no help, in identifying *Brucella* DNA in SAT- negative but Complemented Standard Agglutination Test (C-SAT) - positive specimens. Its greatest is that it fails to give positive results in a situation where there is no circulating bacterial DNA in the blood stream [46].

Quantitative Real-Time PCR (qPCR): Quantitative Real-Time PCR (qPCR) integrates the roles of detection and quantification and therefore makes it priceless in clinical prognostics. During transportation, entire blood samples were kept at about 4°C, and before analysis, they were kept at -80°C. The axiprep Blood Genomic DNA medium volume kit was used to extract DNA from *Brucella* nucleic acid, and the one-tube nested qPCR assay was used for testing; alongside the traditional qPCR test [47]. According to Liu H et al., (phn), a one-tube nested qPCR was reported with a sensitivity of 98.6% and specificity of 100% in detecting Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2); the test produced significantly lower Cycle threshold (Ct) values compared to standard qPCR, making it possible to identify the SARS-cov-2 virus in samples with low viral loads early [36]. Alirezaei A et al., have used qPCR specific to the sequence IS711 to identify the species level in aborted ruminants, but its use in other matrices of samples like cerebrospinal or joint fluid has not been tested independently, which limits its universal applicability [48].

Droplet Digital PCR (ddPCR): Droplet Digital PCR (ddPCR) is a recent development in *Brucella* testing, as it uses the partitioning of DNA into tens of thousands of droplets to enable the very sensitive quantification of the DNA. Liu X et al., showed that ddPCR had high sensitivity (97.12%) relative to qPCR (88.14%) and had an outstanding specificity (36/100%) [47]. In order to detect *Brucella* DNA using quantitative real-time PCR (RT-Qpcr), 487 whole blood and serum samples from probable human brucellosis were collected. In addition, Liu H et al., indicated that ddPCR improved chronic or seronegative-based detection especially in situations where conventional methods are not effective [36]. The same study also noted that ddPCR had the ability to identify *Brucella* DNA in 57.6% of the SAT-negative cases and it gave quantitative data to treat relapse. However, some limitations still exist; there is no confirmation of culture in the technique, and it might need clinical interpretation since it is very sensitive.

CRISPR-Cas-Based Diagnostics: CRISPR-Cas12-based diagnostics is also being hailed as a breakthrough technology in the detection of *Brucella*. Recently, Dang S et al., described a fast test based on CRISPR-Cas12a that detects *Brucella* DNA in 30 minutes with a detection of 10 copies/μL. The assay was highly specific, and no cross-reactivity was reported, and it was superior to RBPT in sheep and cattle. Liu H et al., took this one step further and combined CRISPR-Cas13a with Recombinase-Aided Amplification (RAA) and achieved 93% positivity in milk and 82.1% in blood samples [36]. Although these methods provide significant speed and portability, they are not highly validated in various field conditions and depend on preamplification.

Multiplex PCR (AMOS-PCR): In the study by Waringa NMA et al., have used multiplex PCR, hereafter referred to as AMOS-PCR, to detect *Brucella abortus* as to well as distinguish it with other species [49]. Although both FBAT and RBPT had varied prevalence outcomes, the PCR was the only method that gave clear identification of the intended species. The assay was useful in terms of species-level resolution, but it can only be applied on a small proportion of the samples, which restricts the use of the data in terms of epidemiological meaning.

[Table/Fig-1] summarises all the studies related to conventional and molecular methods in humans [3,25,28,29,31,33,38,45,46,47,49-56] and [Table/Fig-2] depicted the animal studies [30,50,57-64].

S. No.	Author, reference	Year	Conventional (Serological) diagnostics	Molecular diagnostics	Sensitivity/Specificity	Limitations
1.	Rahbarnia L et al., [45]	2020	SAT, C-SAT, 2-ME and blood culture methods	Nested PCR	Nested PCR showed highest sensitivity/ specificity; superior to SAT, serum PCR, and culture.	Blood culture is slow and insensitive; serology may miss chronic cases; PCR sensitivity is limited, especially in serum or absent DNA.

2	Dong SB [3]	2019	FPA, ELISA, RBT, SAT, Coomb's		Fluorescence Polarisation Assay (FPA): 94.5% sensitivity, 100% specificity; FPA+ELISA: 98% sensitivity, 100% specificity.	No bacterial culture performed; FPA reproducibility not validated.
3	Lukambagire AS et al., [51]	2021	RBT 1:2, RBT 1:8, cELISA, Amitech, Arkray, Eurocell, Fortress		Rose Bengal Test (RBT) 1:8 showed highest accuracy and specificity; competitive ELISA (cELISA) high sensitivity; commercial agglutination tests low accuracy.	Small sample; limited generalisability; lab-based tests; imperfect case definitions; commercial kits rarely used as recommended.
4	Almashhadany DA et al., [28]	2022	Rose Bengal Test, Blood culture		Prevalence: 12.3% probable, 9.5% confirmed; RBT accuracy: 100% sensitivity, 96.9% specificity; Isolates: *B. melitensis* (58.1%), *B. abortus* (41.9%).	Few risk factors studied; recall bias; no advanced molecular diagnostics.
5	Liu L et al., [52]	2023		One-tube nested quantitative real-time PCR (qPCR)	Sensitivity 98.6%, Specificity 100%; ~100× more sensitive than qPCR; lowers CT by 6.4 in low-load samples.	Tested only in blood; performance in other fluids unknown; broader validation needed.
6	Feng Q et al., [38]	2025	Blood culture, general bacterial culture, RBT, SAT, C-Reactive Protein (CRP) detection		Blood culture (78.8%); RBT + SAT sensitivity 96.2%; CRP cut-off 1.23 mg/mL (72.7% sensitivity, 71.8% specificity, AUC 0.765); complications: lumbar spine 41.5%, knee 12.8%, meningitis 0.9% mortality.	Non blood culture methods underused; limited pathogenesis studies; antimicrobial susceptibility testing not routine.
7	Liu X et al., [47]	2023	(RBT and SAT)	Droplet-based Digital PCR (dd-PCR), Quantitative Real-Time PCR (RT-qPCR),	dd-PCR showed higher sensitivity (97.1%) and specificity (100%) than RT-qPCR (88.1%, 100%), improving detection in chronic or false-negative *Brucella* cases.	No culture gold standard; cross-sectional design; dd-PCR requires clinical correlation.
8	Waringa NMA et al., [49]	2023	- Febrile *Brucella* Antigen Test (FBAT) - Rose Bengal Plate Test (RBPT)	Multiplex PCR	Brucellosis positivity: 26.5% (FBAT) vs 10.2% (RBPT); FBAT showed low (79.2%) and Positive Predictive Value (PPV) (29.5%); only *B. abortus* detected by PCR.	Hospital-based, cross-sectional; limited PCR testing; children excluded; unvalidated FBAT; female majority; potential recruitment bias.
9	Lu J et al., [53]	2023	Vertical Flow Immunoassay (VFIA)		Limit of Detection- 0.1 IU/mL; Sensitivity 98.3%, Specificity 100%, Accuracy 99.2%.	Cross-reactivity with *Y. enterocolitica* O9; long-term storage not validated.
10	Golchin M et al., [25]	2022	Indirect ELISA		Sensitivity 100%, specificity 94%, AUC 0.985; accuracy comparable to commercial LPS ELISA (94%).	4 false positives; cross-reactivity; needs larger cohort validation; single-antigen limits specificity.
11	Barkay O et al., [29]	2024	Rose Bengal Test (RBT)		Sensitivity 88.3%; rapid screening tool with some false negatives in acute cases.	False negatives in acute phase; observation-based and subjective.
			Coombs Gel Test (CGT)		Sensitivity 83%; effective for detecting blocking antibodies, especially in chronic cases.	False negatives in acute cases; titer-dependent results
			ELISA		Sensitivity 89.4%; highly effective in acute phase; ELISA positivity linked to spondylodiscitis.	Small sample for organ-specific evaluation; limited outpatient availability.
			Blood culture		Sensitivity 34.8%, specificity 100%; gold standard but less sensitive in chronic/subacute cases.	Time-consuming, low yield, affected by prior antibiotics, labour-intensive.
12	Loubet P et al., [33]	2024	Rose Bengal Test (RBT), Standard Agglutination Test (SAT), *Brucella*capt, ELISA IgM & IgG		RBT combined with *Brucella*capt and ELISA (IgM/IgG) showed 90.5% sensitivity, 99.7% specificity, 92.4% PPV, 99.6% Negative Predictive Value (NPV).	SAT discontinued; serology has low PPV, false positives, and stage-dependent variability.
13	Freire ML et al., [31]	2024	Rose Bengal		High sensitivity (96.6%) and specificity (97.9%)	Very low certainty of evidence; influenced by case selection bias
			IgG/IgM ELISA		High sensitivity (96.8%) and specificity -98.6%_	High heterogeneity; healthy controls used; poor antigen description
				PCR	Moderate sensitivity (79.6%) and high specificity (96.0%)	Affected by timing, DNA target variability, and prior antibiotic treatment
			SAT		Sensitivity 89.2%, specificity 95.6%	Suboptimal specificity; lacks interpretive standardisation
			Coombs Test		Sensitivity 89.4%, specificity 98.8%	Limited number of studies; high heterogeneity
			IgM ELISA		Sensitivity 55.3%, specificity 96.8%	Lower sensitivity; possibly phase-dependent performance
			IgG ELISA		Sensitivity 94.4%, specificity 98.5%	Based on limited data; wide confidence intervals
			Immunochromatographic		IgM Sensitivity 70.6%	Low accuracy; specificity data lacking; poorly validated for widespread use
14	Xie Y et al., [46]	2025	-ELISA using BP26 mab (monoclonal antibody)		Achieved 100% sensitivity and specificity for human brucellosis, outperforming LPS C-ELISA (Sens 76.8%, Spec 66.3%).	Small sample; limited validation; no culture comparison or cost-effectiveness analysis; real-world testing and cross-reactivity not fully addressed.
			I-ELISA indirect Enzyme-Linked Immunosorbent Assay)		Fusion protein: sensitivity 90.6%, specificity 94%, AUC 0.959; lower cross-reactivity than LPS, no live *Brucella* needed.	Cross-reactivity still observed (20% in non-*Brucella* febrile cases); Prot

15	Wu Q et al., [54]	2025	Indirect ELISA (iELISA)	TMT-based proteomics, Construction of multiepitope fusion protein	Fusion protein showed high diagnostic accuracy for brucellosis (AUC 0.958, Sensitivity 93%, Specificity 85%), with lower cross-reactivity than LPS antigen.	Limited sample; cannot distinguish vaccine vs. natural infection; 90.1% protein purity; possible *E. Coli* cross-reactivity.
16	Wang Q et al., [55]	2025	Indirect ELISA using multiepitope fusion protein		-High diagnostic performance (Sensitivity 81%, Specificity 99.5%, AUC 0.954) with lower cross-reactivity than LPS and Rose Bengal antigens.	Lower sensitivity than LPS/RBPT; limited, non-diverse sample; not tested against *Y. Enterocolitica* O9 or *E. Coli* O157:H7; epitope/linker not optimised; possible non specific reactions.
17	Mao S et al., [56]	2025		CRISPR/Cas12b-MCDA-LFB	- Detects *Brucella* genus and *B. Melitensis* with high sensitivity/specificity (LOD 2 copies/µl) in ≤90 min, no cross-reactivity, effective on blood, serum, and Cerebrospinal Fluid (CSF).	Multiple Cross Displacement Amplification (MCDA)

[Table/Fig-1]: Comparative summary of conventional and molecular diagnostic methods for *Brucella* detection (human study) [3,25,28,29,31,33,38,45,46,47,49-56].

S. No.	Author, reference	Year	Conventional (Serological) Diagnostics:	Molecular Diagnostics:	Sensitivity/Specificity	Limitations
1	Rodrigues Dos Santos Souza M et al., [57]	2022	Rose Bengal (RB) test		High sensitivity, specificity, and Positive Predictive Value (PPV)—effective screening tool.	Lower specificity; possible false positives in low-prevalence settings; few negative samples.
			Serum Agglutination Test with 2-Mercaptoethanol (SAT/2-ME)		High sensitivity (0.963) and specificity (0.875), comparable to RBT.	Similar to RB; slightly lower specificity; not suitable alone for chronic case confirmation.
			Bacteriology (Isolation)		Moderate sensitivity (0.594) but highest specificity (0.992) among tests	Low sensitivity from ruptured lesions; costly, labour-intensive, and hazardous.
2	Legesse A et al., [30]	2023	RBPT, I(Indirect)-ELISA, CFT	PCR	I-ELISA showed 100% sensitivity and specificity; RBPT had 100% sensitivity in small ruminants but missed some cattle cases; CFT was least sensitive (100% specificity); PCR confirmed *B. abortus*, not *B. melitensis*.	RBPT false positives/negatives; CFT low sensitivity and anti-complementary issues; PCR not performed on all positives.
3	Dang S et al., [50]	2023		CRISPR-Cas12a + RPA + Test strip (CRISPR/CAST)	Rapid on-site *Brucella* DNA detection (30 min, 10 copies/µL) showed high specificity; detected 31/398 sheep and 8/100 cattle, outperforming RBT.	Requires precise temperature control; DNA-only detection; pre-prepared reagents needed; lower signal than qPCR.
4	Freddi L et al., [58]	2023	Lateral Flow Immunochromatography Assay (LFIA)		High concordance with World Organisation for Animal Health (WOAH) tests (RBT, CFT, iELISA); sensitivity and specificity >97%; suitable for field use; PPV 91–100%.	No confirmed *Brucella*-free reference; LFIA had rare false results, batch variation, and low-intensity bands.
5	Wu Q et al., [59]	2024	Gold Immunochromatographic Assay (GICA)		Detected smooth *Brucella* (*B. melitensis*, *B. abortus*, *B. suis*) with high specificity, moderate sensitivity; lods: *B. melitensis* 7.81×10^5, *B. abortus* 3.13×10^6, *B. suis* 1.56×10^6 CFU/mL; no cross-reactivity.	Lower sensitivity than qPCR; not validated on diverse samples; some cross-reactivity with *Ochrobactrum anthropi*.
6	Ahangari A et al., [60]	2024		Aptasensor using gold nanoparticles and DNA aptamer with colorimetric reaction (red to purple)	LOD 1.5×10^1 CFU/mL for *B. melitensis*; faster and more sensitive than PCR (LOD 1.5×10^5 CFU/mL).	Lower sensitivity in real samples; specificity limited to *B. melitensis* and *B. abortus*; not validated in large-scale field settings.
7	Di Febo T et al., [61]	2025	RBT (homologous and heterologous antigens), c-ELISA (s-LPS antigens)		RBT [homologous] Sens 80%, Spec 44.1%, Acc 46.9%; RBT (heterologous0 Sens 80%, Spec 17%, Acc 21.9%; c-ELISA Sens 66.7%, Spec 97.3%, Acc 95%. Best practice: RBT (homologous) + c-ELISA in parallel.	Haemolysed samples; false positives/negatives from poor quality or degradation; limited validation for cetaceans
8	Wang B et al., [62]	2025		Time-Resolved Fluorescence Immunochromatographic Test Strip (TRFICTS)	Ultra-sensitive LOD 0.048 IU/mL (12,800× RBT); portable, rapid 30-min test; high specificity and repeatability; detects weak positives missed by ELISA/GICA.	Cannot distinguish vaccine vs. Wild-type strains; low throughput unsuitable for batch screening.
9	Lecuyer TE et al., [63]	2025	ELISA		Highest sensitivity: 96.9% (vs 2ME-RSAT); high agreement (κ=0.87).	Low specificity (64.5–79.3%); cross-reactivity with other Gram-negative bacteria.
			Veterinary Medical Research and Development (VMRD) *Brucella canis* Indirect Fluorescent Antibody (IFA)		High sensitivity (88–100%), specificity (≤97.5%), and excellent interrater agreement (κ=0.92).	Lower sensitivity than ELISA/LF; false positives at 1:50; subjective result interpretation."
10	Vection S et al., [64]	2025	RBT, iELISA, CFT, Culture	PCR, qPCR, MLVA, Sequencing, Mass spectrometry	Most studies relied on serology (RBT, iELISA); only 16% followed WOAH guidelines, 28.6% used direct methods, highlighting gaps in diagnostics and compliance.	

[Table/Fig-2]: Comparative summary of conventional and molecular diagnostic methods for *Brucella* detection (animal study) [30,50,57-64].

CONCLUSION(S)

Brucellosis has remained a significant public-health problem, particularly in the endemic areas where diagnostic facilities are limited. This review follows the historical development of *Brucella* detection with emphasis on the transition of classical serology and culture-based assays to highly specific and sensitive molecular and biosensor-based assays. Traditional procedures, namely the RBPT, the SAT, and the ELISA, remain essential screening procedures, especially in resource-constrained settings, since they are simple to operate and inexpensive. However, these methods lack sensitivity and specificity of positive diagnosis and cannot distinguish reliably between acute, chronic or past infections.

Molecular diagnostic methods, in particular PCR, qPCR, and ddPCR have significantly increased sensitivity and time-to-result but their widespread use is limited by technical infrastructure requirements and cost. The latest advancements such as CRISPR-Cas-based diagnostic lateral and RBTs, and recombinant antigen-based ELISAs are expected to overcome these obstacles by bringing together portability, rapid throughput, and increased specificity. However, these state-of-the-art methodologies still need significant field testing and standardisation, before they can be used in more diverse clinical and public-health settings.

Future research must prioritise the development of quick, cheap and field-friendly point-of-care diagnostics, and in particular, in endemic and resource-limited settings. The comprehensive management and control of brucellosis require adoption of a one health framework which involves human, animal and environmental aspects. The common protocols, multicentric assessments, and the combination of new biomarkers will play a vital role in the development of diagnostic potential. Finally, a concerted international initiative on innovation, validation, and accessibility will be needed to reduce the long-term impact of brucellosis on the population.

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