Diagnosis of Human Brucellosis
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Abstract

Brucellosis is a worldwide zoonosis with a high degree of morbidity in humans. The disease may be overlooked and misdiagnosed because of the difficult diagnosis and the absence and lack of experience with laboratory testing. As the clinical picture of human brucellosis is fairly non-specific, a definitive diagnosis requires isolation of the causative organism, or the demonstration of the high levels of specific antibodies, or organism specific DNA in samples. Isolation of organism is time-consuming and hazardous, so must be performed by highly skilled personnel. So serological methods are preferred. Advanced serological methods like ELISA, Brucellacap are more sensitive techniques. Combination of culture and serological test should be used to avoid misdiagnosis. Molecular assays come with high sensitivities and specificities and may reduce diagnostic delays in clinical laboratories. Several PCR based assays have been studied for standardizing them for brucellosis diagnosis. Another rapid, sensitive and inexpensive molecular technique, LAMP, has been developed by designing primers specific for Brucella abortus genes. This technique can prove helpful in resource limited settings in developing countries. Highthroughput MLVA-16 genotyping techniques are also being studied and developed for brucella typing for tracing the source of brucellosis infection. This test can be useful for brucella diagnosis and surveillance studies. This review article describes procedures, advantages or limitations of some useful conventional diagnostic techniques to more sophisticated molecular techniques for brucellosis diagnosis.

Keywords: Brucellosis; Diagnosis; PCR; LAMP; MLVA-16 typing

Introduction

Brucellosis is a worldwide zoonosis with a high degree of morbidity in humans. According to WHO data about 500,000 cases of this disease are registered in the world every year [1,2]. Brucellosis remains an uncontrolled problem in regions of high endemicity such as the Mediterranean, Middle East, Africa, Latin America and parts of Asia [2,3]. The presence of brucellosis in India was first established early in the previous century and since then has been reported from almost all states [4]. It is mainly transmitted from cattle, sheep, goats, pigs and camels through direct contact with blood, placenta, fetuses or uterine secretions, or through consumption of contaminated raw animal products (especially unpasteurized milk and soft cheese). Furthermore, brucellosis is the most common bacterial laboratory-acquired infection worldwide [5].

The disease primarily presents as fever of unknown origin with multiple clinical signs and symptoms. Patients regularly suffer serious focal complications such as spondylitis, neurobrucellosis or Brucella endocarditis [6]. The clinical features and presentation of human brucellosis overlap with many other infectious and non-infectious diseases [7] such as typhoid fever, rheumatic fever, spinal tuberculosis, pyelitis, cholecystitis, thrombophlebitis, autoimmune disease, and tumours [8-10]. The clinical picture is not specific and laboratory testing should support the diagnosis. A proper diagnosis is important, as therapeutic failure and relapse, a chronic course, and sometimes severe complications such as bone and joint involvement are characteristic of the disease [11]. To an unaware physician, the diagnosis of brucellosis can be problematic. Questioning the patient at this stage about animal contacts and food habits could be helpful to raise suspicion of brucellosis when either the patient admits to own or work with livestock and mentions signs of brucellosis such as hygromas, infertility or abortions in his animals, or if the patient has a taste for fresh unpasteurized dairy [1].

Presumptive diagnosis of brucellosis can be made by the use of several serological tests to Brucella antibodies, but the “gold standard” remains isolation and identification of the bacterium. However, cultural examinations are time-consuming, hazardous and not sensitive. Despite the vigorous attempt for more than one century to come up with a definitive diagnostic technique for brucellosis, diagnosis still relies on the combination of several tests to avoid false negative results [12].

Laboratory Diagnosis

Serological tests

Serum agglutination test: Serum Agglutination Test (SAT) is performed by mixing serial dilutions of serum, usually 1:20 through 1:2,560, with Brucella antigen in test tubes or in wells of an ELISA plate. After overnight incubation agglutination is read either by the unaided eye or under a binocular. As a guidance agglutination at titres of 1:160 or above is considered of diagnostic value as long as the patient has signs and symptoms of disease. In endemic areas, the diagnostic threshold value will have to be set at least one titre step higher (1:320) to provide a sufficiently high specificity as many asymptomatic individuals will have titres equal to the lower threshold level of 1:160 [13]. Sometimes SAT is performed in the presence of the reducing agents 2-mercaptoethanol (2-ME) or dithiothreitol (DTT). These reducing agents destroy the agglutinating activity of immunoglobulin M (IgM) leaving IgG intact [11]. The 2-ME or SAT-DTT test is used to increase the specificity of the reaction by looking at IgG only, which is important in patients with a more persistent infection [14]. Either reducing agent may be added to serum as a diluent, using dilutions of 1:25 and increasing. For the reaction of brucellosis, reaction at a 1:25 serum dilution is considered positive. Some false negative reactions occur as some IgG molecules are also susceptible to reduction of disulfide bridges, rendering them

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unable to agglutinate, however, in general, reduction of IgM increases specificity. Care must be taken when using 2-mercaptoethanol as it is toxic and should only be used in a well-ventilated area or a chemical hood [15].

Although the definite cure of Brucella infections is usually associated with lower SAT titres, significant titres can be found in 3% to 5% of patients up to two years after successful antibiotic treatment [16]. SAT suffers from high false-negative rates in complicated and chronic cases. SAT titers ≥1:160 are generally considered consistent with active brucellosis if accompanied by a compatible clinical course in patients with a history of potential exposure. In the early course of the disease, even bacteremic patients may present with titers ≤1:160 [17, 18].

Rose Bengal test: The Rose Bengal Plate Agglutination test (RBT) is a rapid test which was designed originally for screening use in veterinary medicine, but is now often used for the diagnosis of human brucellosis [19-21]. Its high sensitivity, ease and speed of use, as well as its low cost, have made it very popular in hospital emergency departments for the diagnosis of febrile syndromes [22]. For this test 30 mL of plain serum is dispensed on a white glossy ceramic tile and mixed with an equal volume of RBT antigen (previously equilibrated at room temperature and shaken to resuspend any bacterial sediment) using a toothpick. The tile is then rocked at room temperature for 8 minutes (instead of the 4 minutes recommended for animal brucellosis) and any visible agglutination and/or the appearance of a typical rim is taken as a positive result [7]. Positive sera are tested further as follows: Eight 30 mL drops of saline are dispensed on the tile and the first one is mixed with an equal volume of the positive plain serum (1/2 serum dilution). Then, 30 mL of this first dilution are transferred to the second drop with the help of a micropipette and mixed to obtain the 1/4 dilution. From this, the 1/8 to 1/128 dilutions are obtained by successive transfers and mixings taking care of rinsing the pipette tip between transfers. Finally, each drop is tested with an equal volume (30 mL) of the RBT reagent, so that the final dilutions ranges from 1/4 to 1/256 [23,24].

Ruiz-Mesa et al. [22] conducted a study in which serum of individuals with no regular exposure to or history of brucellosis, individuals exposed repeatedly to Brucella infection and individuals infected with Brucella who had received appropriate treatment during the previous 12 months was compared in which specificity of the Rose Bengal Test was 94.3%, 91.7% and 76.9%, respectively for the three groups. The overall sensitivity of the test was 92.9% subjected serum of patients exposed to brucellosis to Rose Bengal plate test and analysed by complement fixation test (CFT). The sensitivity and specificity of RBPT were 78.3% and 81% respectively taking CFT as gold standard. The test is simple to perform, rapid (within 5-10 min) and has relatively good results in diagnosing patients with acute brucellosis, but gives a high rate of false-negative results in chronic and complicated cases. No single test is perfect, clinical history coupled with combination of two or more tests reduces diagnostic errors [25,26].

Lateral flow assay: For the lateral flow assay, 5 μl of patient serum followed by 130 μl of running fluid are added to the sample application pad in the sample well of the plastic assay devise [23]. The assay is read after 10 to 15 minutes by visual inspection for staining of the test and control lines in the assay window of the device. The test line may stain at different intensities and is subjectively rated 1+ when staining is weak, 2+ when staining is moderate, 3+ when staining is strong, and 4+ when staining is very strong. The test is scored negative when only staining of the control is observed [18]. Memish et al. [18] subjected the samples of patients who presented with clinical suspicion of brucellosis over a one-year period to lateral flow assay to assess the diagnostic value of this test. The sensitivity of the lateral flow assay calculated for the Brucella IgM/IgG was 95% and specificity was 97%. This test is simple and easy to perform.

Compliment fixation test: Complement fixation test is a widely used confirmatory test for brucellosis. The basic test consists of B. abortus antigen, usually whole cells, incubated with dilutions of heat inactivated (to destroy indigenous complement) serum and a titrated source of complement, usually guinea pig serum. After a suitable time a pretitrated amount of sheep erythrocytes coated with rabbit antibody is added. If a primary immune complex (B. abortus cells and test serum) is formed due to the presence of certain antibody isotypes in the serum, complement was activated and therefore not available to react with the secondary immune complex of sheep erythrocytes and rabbit antibody, resulting in no or only slight lysis of the erythrocytes. Alternately, if no primary immune complex was formed, complement would cause all the sensitized sheep erythrocytes to lyse. Thus the amount of haemoglobin in solution is an inverse measure of anti-Brucella antibody activity.

Coombs antiglobulin agglutination test: For this test, serial dilutions of the patient's serum are prepared in saline making final volume of the diluted serum in each tube 1 ml. 1 ml of B. abortus antigen, ATCC 11192 [27] is added to each tube. After mixing, incubation is carried out at 37°C for 24 hours and at 5°C for 1 hour. The tubes are then centrifuged at 4,000 r.p.m. for 15 minutes. The supernatant is discarded. 2 ml of 0.85 per cent saline solution is added to the sediment. After the sediment is resuspended by shaking, the tubes are again centrifuged. The washing of the sediment is carried out three times and the sediment is then resuspended in 0.5 ml of saline. Coombs anti- humanoglobulin rabbit serum (0.1 ml) is then added to each tube as well as a control tube with no immune serum. After mixing, incubation is carried out at 37°C for 30 minutes. Agglutination is then recorded [28].

Serra and Vinas [27] analysed sera obtained from brucellosis patients with primary infection(group1) and patients with previous infection (group2) using the rose Bengal test, standard serum agglutination test (SAT), Coombs' test, ELISA, and complement fixation test. Blood culture was valuable in group1 patients but inappropriate in group2. The combination of positive rose Bengal test and Coombs' test ≥ 1/320 was the best diagnostic criterion with 80% specificity and 100% sensitivity. Coombs' test may be more suitable for confirmation of brucellosis in relapsing patients or patients with persisting disease.

Enzyme linked immuno sorbent assay: The enzyme linked immunosorbent assay (ELISA) is known as a sensitive and rapid method for diagnosis of brucellosis. Detection of specific immunoglobulin by a single, simple and rapid test is a major advantage with ELISA [29,30,7]. In addition to benefit of ELISA in diagnosis of brucellosis in endemic area, it could be useful as a screening test in areas with low incidence of disease [31]. Blood samples are obtained from all patients and controls and checked for Brucella IgM and IgG antibodies by ELISA test using commercially available ELISA kits according to the standard procedure. In one study by Memish et al. the sensitivity of SAT for diagnosis of brucellosis was similar to combination of IgM and IgG ELISA test [32]. In another study, Cifci et al. found the sensitivity 94.3%, 97.1%, and 71.4% for SAT, ELISA IgG and ELISA IgM, respectively [33]. Hasibi et al. investigated the accuracy of ELISA test for diagnosis of human brucellosis and determined the optimal cut-off value for ELISA results. ELISA IgG test was more reliable than ELISA IgM test in diagnosis of human brucellosis. Using a cut-off of 10 IU/ml and 50 IU/ml had most sensitivity (92.9%) and most specificity (100%) for ELISA IgG test [34].

Immunocapture-agglutination test (brucellacapt): Brucellacapt
is a single stage proprietary test kit and is a new form of the agglutination test to test for brucellosis antibodies in sera. In this test, 0.050 ml samples of serum dilutions were added to wells of a U-bottom microtiter plate coated with anti-total human immunoglobulin. Then 0.050 ml of an antigen suspension (colored B. melitensis bacteria killed by formaldehyde aldehyde treatment) was added to all the wells. The plates were sealed with adhesive tape and incubated at 37°C for 24 h in a dark humid chamber. Positive reactions show agglutination over the bottom of the well. Negative reactions are indicated by a pellet at the center of the bottom of the well [34]. In a study conducted by Orduna et al. [35] for evaluating the validity and the usefulness of brucellacapt test for the diagnosis of human brucellosis, 95.1% sensitivity and 99% specificity was found. The diagnostic efficiency of brucellacapt was 0.987852 with 95% confidence interval. This study showed that the Brucellacapt and Coombs tests have very similar performances in the diagnosis of human brucellosis. Brucellacapt is more sensitive and usually shows higher titers than the Coombs test.

Culture

Conventional culture technique

Culture from the blood of a patient provides definite proof of brucellosis [36]. There is a range of commercially available culture media for growing Brucella. The most common basal media in use are: Tripticase soy (BBL®), Bacto Tryptose (Difco®), Tryptic soy (Gibco®), Tryptone soya (Oxoid®). The powder media can be used to prepare either broth or agar medium. For culturing blood and other body fluids, it is preferred to use broth or a biphasic medium (Castaneda), mainly because Brucella is often present in small numbers. For other specimen, solid media with 2.5% agar facilitate the recognition of colonies and discourage bacterial dissociation. The Castaneda two-phase system is the most convenient. It consists of a bottle which contains both solid and liquid medium with 1-2% sodium citrate in the liquid phase. An inoculum of 5-10 ml is added to the bottle and incubated at 37°C in the upright position in a closed jar or incubator in 10% carbon dioxide (CO₂) atmosphere. If no colonies are observed on the surface of the agar, the bottle should be tilted every 24-48h to allow the broth flow over the agar. Positive cultures may be evident within one or two weeks. It is advisable not to discard cultures as negative until four to six weeks have elapsed. When colonies are present they should be subcultured for further examination and typing [37].

Semi automated blood culture technique

With the launch of automated blood culture systems, such as the BACTEC™ (Becton Dickinson Diagnostic Systems, Sparks, Maryland, USA) and the BacT/Alert™ (bioMérieux Inc., Durham, North Carolina, USA), which continuously monitor the CO₂ release of potentially growing microorganisms, and the BACTEC™ Myco/F-Lytic system (Becton Dickinson Diagnostic Systems), which integrates lytic activity and automation [30], the time to detection has been significantly reduced. Brucelae can be detected in the blood of infected patients after four days of culture or less [38].

Lysis centrifugation blood culture technique

The LC technique uses lysis of erythrocytes in a citrate solution, followed by isolation of Brucella bacilli by centrifugation of the sample, which concentrates the bacilli and facilitates growth after subsequent plating [17]. For lysis centrifugation technique, a 5-ml aliquot of blood drawn simultaneously along with that used for the Castaneda culture is added to a 50-ml screw-cap sterile centrifuge tube containing 20 ml of sterile distilled water and 1.5 ml of 4% sodium citrate. The contents are gently mixed, and the tube is centrifuged at 2,000 × g for 30 min. The supernatant is discarded, and the sediment is inoculated onto brain heart infusion agar plates in duplicate. The plates are incubated at 37°C with and without carbon dioxide for 7 days. The bottles and plates are observed daily. The date of the appearance of the first colony is recorded. [38,39]. Porter-Jordan et al. [40] have also reported the rapid recovery of brucelae within 48 h in their study of the lysis centrifugation procedure. Pappas et al. [30] studied that the lysis concentration technique has not only detected the pathogen earlier but also picked up a larger number of cases (20% more acute and 40% more chronic cases. They reported that they would have missed 34 cases if conventional culture alone had been performed.

Clot culture technique

In this method, the blood clot preserved in the sterile screw capped plastic tube with glass beads after removal of serum is used for this method. The clot is disrupted by shaking the tubes on a shaker for 15 min. The disrupted clot is then inoculated in Castaneda’s medium and incubated at 37°C with 10% CO₂, for a maximum of 30 days [41].

Identification of Brucella strains was done using standard classification tests, including Gram staining, a modified Ziehl-Neelsen stain, growth characteristics, oxidase activity, urease activity, H₂S production (4 days), dye tolerance such as basic fuchsin (1: 50 000 and 1: 100 000) and thionin (1: 25 000, 1: 50 000 and 1: 100 000) and B. abortus and B. melitensis monospecific antisera (Murex Biotech) were used for seroagglutination test [42].

Molecular Techniques

PCR based assays

Direct detection of Brucella DNA in brucellosis patients is a challenge because of the small number of bacteria present in clinical samples and inhibitory effects arising from matrix components [43]. Polymerase chain reaction (PCR) assays can be used to amplify and detect Brucella DNA in pure cultures and in clinical specimens. The QIAamp™ DNA Mini Kit (Qiagen Inc., Valencia, California, USA) and the UltraClean™ DNA BloodSpin Kit (MO BIO Laboratories Inc., Carlsbad, California, USA) are among the many commercial kits that have been successfully used to extract Brucella DNA from whole-blood, serum and tissue samples [44].

Standard PCR

For the diagnosis of human brucellosis, a PCR assay with one pair of primers was developed, which amplifies the target genomic sequence of Brucella species. Studies showed that standard PCR appeared to be a more sensitive technique than microbiological methods, not only for the diagnosis of a first episode of infection, but also for the early detection of relapses [45-47]. Baddour et al. compared sensitivity of 3 pairs of primers amplify 3 different fragments including a gene encoding BSCP 31 (B4/B5), a sequence 16s rRNA of B. abortus (F4/R2), and a gene encoding omp2 (JPF/JPR). The results showed that the sensitivity of the B4/B5 primer pair, JPF/JPR primer pair and F4/R2 primer pair was 98%, 88.4% and 53.1%, respectively [48]. Navarro et al. [49] also compared PCR methods using these 3 pairs of primers as described above. Their results further indicated that the three primers assayed showed a difference in sensitivity by the presence of human genomic DNA.

Real time PCR

Real-time PCR is a valuable technique in quantification of nucleic
of the loop primers (LF and LB), 1.4 mmol/L each deoxynucleotide (FIP and BIP), 5 p/mol of each outer primers (F3 and B3), 20 p/mol per 25 µl volume containing 40 p/mol of each inner primers biovars but B. melitensis primers and enzymes are able to differentiate Brucella species and biovars, with a view to epidemiology of brucellosis, recognition of Brucella species and biovars, and accurate diagnosis of acute human brucellosis [54].

Nested and Semi-nested PCR

The nested PCR means that two different pairs of PCR primers are used for a single locus [40]. Semi-nested PCR has two different pairs of PCR primers, but the second pair of primers has one primer identical to the first pair [53]. Nested PCR and Semi-nested PCR assays are now developed for identifying Brucella in samples of human blood and then to explore their clinical practice for the diagnosis of human brucellosis. Lin et al. reported a nested PCR for the laboratory diagnosis of human brucellosis [54].

Other PCR based assays

Vrioni et al. employed a simple Polymerase Chain Reaction-Enzyme Immunoassay (PCR-EIA) for the rapid laboratory diagnosis of human brucellosis directly from peripheral blood. Following the amplification of a 223-bp sequence of a gene that codes for the synthesis of an immunogenic membrane protein specific for the Brucella genus, the amplified product was detected in a microtiter plate by hybridization with specificity of 100% and sensitivity of 81.5% for whole blood specimens and 79% for serum specimens. Results suggest that PCR-EIA assay is a sensitive and specific method that could assist the rapid and accurate diagnosis of acute human brucellosis [55].

Due to differences in the pathogenicity of Brucella species and biovars, with a view to epidemiology of brucellosis, recognition of Brucella biobvars is important and hence typing of various strains is the main task of control centers of brucellosis and must be performed continuously. Minzred et al. used PCR-Restriction Fragment Length Polymorphism Assay (PCR-RFLP) for molecular typing of Brucella abortus and Brucella melitensis. Results of the study showed that the primers and enzymes are able to differentiate B. melitensis biobvars but could not distinguish all of the B. abortus biobvars from each other [56].

Loop- mediated isothermal amplification assay

Loop- Mediated Isothermal Amplification Assay (LAMP) is performed in 25 µl volume containing 40 p/mol of each inner primers (FIP and BIP), 5 p/mol of each outer primers (F3 and B3), 20 p/mol of the loop primers (LF and LB), 1.4 mmol/L each deoxynucleotide triphosphate, 1 mol/L betain , 20 mmol/L Tris–HCl (pH 8.8), 10 mmol/L KCl, 10 mmol/L (NH2)SO4, 8 mol/L MgSO4, 0.1% Triton X-100, 25 mmol/L MgSO4, 8 U of Bst DNA polymerase large fragment and 2 µl of template genomic DNA. In addition, 25 µmol/ L calcein as a fluorescent metal indicator is added to reaction. The mixture is incubated at 63°C for 60 min in a Loopamp real-time turbidimeter , in which turbidity readings are obtained in the reaction mix at 650 nm every 6 s. Finally, the reaction is terminated by heating at 80°C for 5 min. The LAMP reactions are examined by electrophoresis of products on 2% agarose gel and direct visual observation to judge turbidity or colour changes. For the specificity confirmation of the LAMP assay, the amplified product is digested with MboI restriction enzyme at 37°C for 7 h and is then electrophoresed on 2% agarose gel [41].

The LAMP assay is advantageous because of its simple operation, rapid reaction and easy detection [41]. A simple and inexpensive apparatus such as a water bath or heat block that provides a constant temperature of 63°C is sufficient for the assay, and, unlike PCR, the reactivity is directly observed with the naked eye needing the neglect for electrophoretic analysis. Moreover, the LAMP assay can be performed on site, as special equipment such as a thermal cycler is not required. Queipo-Ortuno et al. [41] reported that the sensitivity of Brucella LAMP assay was almost equal to that of real-time PCR. Nonspecific amplification was observed in V. cholerae 01 when amplified for more than 42 cycles in real-time PCR, but not in the LAMP. Therefore, the specificity of the LAMP assay was superior to that of real-time PCR [57]. The LAMP method because of the simplicity, low cost (cost per sample is about 3-6 times cheaper than PCR and real-time PCR) and no need for complex equipment, can be preferred to other molecular methods. Also, the LAMP assay for the quantitative detection of Brucella spp. was highly sensitive and specific. Therefore, this method could be a useful tool for rapid detection of Brucella spp. in epidemiologic studies and in resource limited settings in developing countries [58].

Multiple Locus VNTR Analysis (MLVA) typing for Brucella based on microfluidics technology

The strain and biovar typing of Brucella field samples isolated in outbreaks is useful for tracing back source of infection and may be crucial for discriminating naturally occurring outbreaks versus bioterrorist events, being Brucella a potential biological warfare agent. The MLVA band profiles may be resolved by different techniques ranging from low cost manual agarose gels to the more expensive capillary electrophoresis sequencing systems. The most frequently used method is the agarose gel. Recently, a more rapid and inexpensive method based on the Lab on a chip technology has been proposed. This miniaturized platform for electrophoresis applications is able to size and quantify PCR fragments, and was previously used for studying the genetic variability of Brucella spp. [59]. Recently a new high throughput microfluidics system, the LabChip 90 equipment (Caliper Life Sciences), was developed. This platform can be considered particularly useful when dealing with a large number of samples in short time.

First of all, DNA is isolated from the samples positive for brucellosis which have to be genotyped. VNTR amplification is done using PCR utilizing 16 Brucella primers [60]. For MLVA-16 analysis, the amplification is performed in 96-well or 384-well PCR plates. The chip is prepared according to the manufacturer recommendations. Each chip contains 5 active wells: 1 for the DNA marker and 4 for gel-dye solution. For each run a strip well is also prepared with the ladder (containing eight MW size standards of 100 300 500 700 1100 1900 2900 4900 bp) that is inserted into the appropriate groove of the instrument. The number of samples per chip preparation is 400, equivalent or four 96-well plates or one 384-well plate. After gel preparation, the sample plate is loaded into the plate carrier attached to the robot of the Caliper LabChip 90. During the separation of the fragments, the samples are analyzed sequentially and electropherograms, virtual gel images and table data are shown. Amplification product size estimates are obtained by using the LabChip GX. The software allows importing the data to a spreadsheet software and subsequently to the conversion table that allows to assign each size to the corresponding allele. The
maximum and minimum value of the observed sizes for each allele is thus established experimentally. The PCR amplicons are purified and sequenced by automatic DNA Analysis System [37].

Conclusion

Definitive diagnosis of brucellosis remains a difficult task. No single test is perfect, clinical history coupled with combination of two or more tests reduces diagnostic errors. The combination of positive Rose Bengal test and Coombs’ test or Brucellacap is a good diagnostic criterion with 80% specificity and 100% sensitivity among serological tests. LC technique of blood culture is a very sensitive and productive culture technique which gives faster results than conventional culture technique but it should be done using all safety precautions. Independent of the disease stage, Standard and other novel PCR techniques are more sensitive than blood cultures and more specific than serological tests. Newer molecular diagnostic techniques like The LAMP Method because of the simplicity, low cost (as compared to PCR), sensitivity and specificity can be preferred to other

molecular methods and could be a useful tool for rapid detection of Brucella spp. in epidemiologic studies and in resource limited settings in developing countries. Furthermore, High throughput MLVA-16 genotyping technique is useful for tracing back the source of Brucella infection particularly when dealing with a large number of samples in short time.

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