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**Mahvash Hira Khan**
M.V.Sc (Animal Biochemistry), Department of Animal Biochemistry, ICAR-IVRI, Uttar Pradesh, India

**Shantanu Kodape**
M.V.Sc (Animal Biotechnology), Department of Animal Biotechnology, ICAR-IVRI, Uttar Pradesh, India

**Rajeshwar Khandare**
Research Scholar Ph.D. Department of Animal Biotechnology, ICAR-IVRI, Uttar Pradesh, India

**Aditya Agrawal**
Assistant Professor, Department of Veterinary Physiology and Biochemistry, University - College of Veterinary Science and Animal Husbandry, Rewa, Madhya Pradesh, India

**Anil Gattani**
Associate Professor, Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, Rewa, Madhya Pradesh, India

**Corresponding Author**
**Mahvash Hira Khan**
M.V.Sc (Animal Biochemistry), Department of Animal Biochemistry, ICAR-IVRI, Uttar Pradesh, India

# Laboratory diagnosis of brucellosis

**Mahvash Hira Khan, Shantanu Kodape, Rajeshwar Khandare, Aditya Agrawal and Anil Gattani**

**Abstract**
The diagnosis of brucellosis can follow different procedures. This research addresses those procedures. Brucellosis is a significant zoonosis. Its diagnosis and control need rapid action. Otherwise, it can cause huge reproductive losses in all species of animals. In humans as well, brucellosis is chronic and debilitating disease. Brucellosis in human can cause dysfunction of several organs if not treated in time. Thus, early diagnosis through laboratory testing is necessary for quick detection of the disease in humans as also in the animals, especially domestic animals. Identification of the causative agent is necessary in detecting this disease. That is why definitive diagnosis is mostly followed for the detection of the disease. However, it should be kept in mind that definitive diagnosis that needs isolation of the subject is a time-consuming diagnosis process. At the same time, it should be performed by the skilled personnel only. To overcome this issue in definitive diagnosis of brucellosis, serological tests are mostly preferred. In the diagnosis of brucellosis, serological tests have been advanced quite a bit over the last two decades all over the world. Highly advanced molecular DNA technology is found to be providing accurate results in diagnosis. For the rapid identification of species and strains affecting the subject, several types of PCR-based assays can be performed these days. Today, two types of tests are followed. One is direct tests and other one is indirect tests. In the direct tests, the present of *Brucella* is detected when the clinical signs are obvious. In the indirect tests, primary screening is done to found the subclinical situation of a subject.

**Keywords:** brucellosis, diagnosis, bacteriology, serology, molecular methods

## Introduction
In the last decade of 19th century, brucellosis became an obvious physical issue in the British military base in Malta. It caused substantial mortality among the soldiers in that military base in Malta. Dr. David Bruce, a military doctor, was sent to find what is happening there? Dr. Bruce with the help of a medical team and researchers succeeded in finding the cause of such a high rate of mortality in the British military base. They isolated micrococcus melitensis that is found mainly in the goat milk. Military personnel were found to drink the raw goat milk every day [1, 2]. Later, this bacterium was given the name *Brucella melitensis*. It is the genius that was found in the raw goat milk. Later other species of *Brucella* like *Brucella* Abortus was detected by Bang in 1897 [3]. After that *Brucella* Suis was isolated by Traum [4]. All these three species, *viz*. *B. melitensis*, *B. abortus*, and B. Suis are highly significant in terms of public health and economics. Several other species of *Brucella* such as *B. ceti*, *B.* microti, *B. canis*, *B. ovis*, *B. neotomae*, and *B. pinnipedialis* were found later in different animals including marine mammals that are potential pathogens to humans.

The primary signs of brucellosis are several such as abortion, still birth, orchitis, retained placenta, arthritis in different animals and surging fever in humans. The main problem in the diagnosis of brucellosis is that these types of symptoms are common in many other diseases as well. The epidemiological studies help to some extent in understanding the proneness of the disease. History of any recent contact with any contaminated food or infectious diseases may also help in diagnosis of the disease. After that the presumptive diagnosis can be performed through several serological tests that can detect the antibodies for *Brucella*. However, particular diagnosis is required for the bacteriological demonstration of the pathogen. Hence, the shipment and collection of exact samples always keep the high importance in this realm. The definitive technique is required which is not available yet.

The laboratory diagnosis is to be performed with the help of both direct and indirect methods as soon as the subject shows any clinical signs or the epidemiological study finds the high chance of contracting the disease. Perfect diagnostic procedure is imperative for the successfully controlling the disease and its eradication. The European Union through its directive 2003/99/EC has announced that brucellosis and its primary agents require close surveillance. They have also included it in the list of zoonosis.

The existence of *Brucella* spp. Infection can be established through close monitoring and pathological confirmation. The sample once collected can be tested immediately or preserved for testing sometime later. Samples of aborted materials, vaginal fluids, blood, milk, or carcasses can be used for laboratory testing [5]. Some proven methods are available for differentiating the species of *Brucella* species or its diverse biovars. These methods include $H_2S$ production level, $CO_2$ requirements, serotyping, phage typing, and metabolic properties. However, it is noticed over and again that the inconsistency of a few phenotypic characteristics of some strains of *Brucella* sometimes hinders the identification of the species and biovars. To overcome this issue, these days stable DNA markers are used for the identification of the species and biovars.

**Review of literature**

Doosti and Moshkelani (2011) [6] designed a PCR assay that can act on real time for the identification of the species of *Brucella*. Their PCR assay is also capable of differentiating *B. abortus* and *B. melitensis*. The authors performed their tests on mice tissues targeting the BMEII0466 gene of B. Melitensis and BruAb2_0168 gene of *B. abortus*. The real-time PCR detected higher level of specificity in the targeted tissues over gel electrophoresis. When the same test was performed with IS711 gene using another primer, the researchers obtained similar result [6].

Primers aiming several insertion components of IS711 can also be applied with TaqMan® probes for the detection of *Brucella* genus precisely and differentiating the species such as *B. abortus*, *B. ovis*, *B. canis*, *B. melitensis*, and *B. suis* [7].

As per the testing requirements and required test specificity, MLVA can be performed with the help of 8 loci (MLVA-8, panel 1 markers) [8], 11 loci (MLVA-11, panels 1 and 2A markers) [9], and 16 loci (MLVA-16, panels 1 and 2 markers) [10].

MLST has also been used for the identification of *Brucella* [11]. This a modern testing process dependent on DNA sequencing. It is accurate in differentiating diverse bacterial species and identifying their clonal lineages. Through MLST, isolation is possible through DNA sequencing of several genetic loci. After isolating, they are sequenced on both strands with the help of a DNA sequencer. Here, each gene is assigned elemental sequences. These are alleles that carry unique allelic profile also called sequence type (ST). Such sequence of profiles becomes the identification marker.

Whatmore *et al*. (2007) enlarged PCR 9 distinguishable genome fragments. The authors separated the products applying agarose gel electrophoresis and then they performed sequencing of PCR products. The sequences of all 9 loci were concatenated to create a sequence of 4396bp for each of the available genotypes. The authors detected that all allelic patterns were unique. These were identified as a ST. The authors then used software for phylogenetic examination. It helped to design the trees joining the neighbours. The authors opined that this process was potentially effective for detecting *Brucella*.

**Conclusion**

Diagnosis of brucellosis is not easy. The only effective diagnosis is the finding the causative agent and separating it from the host. Several problems occur in the diagnosis of the species of *Brucella* such as problems in isolating the bacteria, cost of the process, and failure in detecting the causative agent. Molecular biology is offering new ways of laboratory works for detecting the bacteria. It will soon replace the traditional processes. Serological tests for this purpose have advanced a lot since its introduction by Wright and Smith in 1897. The modern assays are found to be more efficient and their accuracy level is higher than the previous ones. However, a perfect test for the diagnosis of brucellosis is yet to be invented. Meanwhile, the invention of vaccine that does not hinder the serological tests has made the disease or its diagnosis more manageable. It seems that the accurate detection of the disease may need several types of tests for detecting the immune response from different aspects.

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