

Brucella

Small coccobacilli (0.5 to 0.7 μm by 0.6 -1.5 μm) with rounded ends (often appear like cocci). Obligately aerobic or microaerophilic.

Fastidious, requiring 5% serum or blood supplement for growth. Some species require an atmosphere containing 5-10% carbon dioxide. Usually slow growing on primary isolation (require 5 to 7 days and sometimes longer). Primary plates should not be considered negative until after 21 days incubation. After 48 hr incubation, colonies are from ≤ 1 mm diam, raised, convex with entire edge. Surface maybe smooth mucoid or rough. Smooth colonies have a glistening moist surface and are translucent with a slightly bluish colour. Mucoid colonies are like thick mucus in appearance and consistency. Same size as smooth but flatter with a dull rather than glistening surface. Viscous and adhere to the surface of the media. *B ovis* and *B canis* are permanently nonsmooth (mucoid). Rough colonies are dull yellow and more opaque. Friable and even brittle when probed with a loop. Rough colonies are found infrequently on primary isolation but may appear with repeated sub-cultivation. *Brucella* species are presumptively identified on the basis of their requirement for carbon dioxide, production of hydrogen sulphide and urease, and inhibition by various concentrations of the dyes basic fuschin and thionin. All species except *B ovis* are oxidase positive and break down glucose oxidatively.

Agglutination tests are also used to distinguish species and biovars. Monospecific *B. abortus* (A) and monospecific *B melitensis* (M) antisera agglutinate cells of the 3 smooth species (*B. abortus*, *B. melitensis* and *B. suis*) and is used to help differentiate their biovars. Rough (R) antiserum agglutinates the 2 rough species (*B. ovis* and *B. canis*).

Brucella abortus

Specimen Collection

Foetuses

Aborted foetuses and membranes usually contain large numbers of brucellae.

Specimens for microscopic examination and culture include stomach contents, liver, spleen and gastrointestinal tract associated lymph nodes from aborted foetuses and full-term calves.

Infected Cattle

It is not always possible to isolate *B. abortus* from infected live animals. Milk, tissues and vaginal specimens are likely to contain only small numbers of organisms. Serological techniques are normally employed to detect infected cattle.

B abortus can be isolated from the milk in about 50% of infected cows. Infection may be present in one or more quarters so it is important that samples are collected from all quarters.

Brucellae may be recovered from vaginal secretions collected up to 6 weeks after abortion or parturition.

Tissues to be collected at necropsy for isolation of *B. abortus* include lymph nodes associated with the alimentary tract, udder and supermammary lymph nodes, and postparturient uterus.

Microscopic Examination of Tissues

Prepare smears of lesions and stain by Gram and using the modified Ziehl Neelsen (MZN) technique.

May see small Gram-negative rods which appear acid fast using the MZN technique.

Cultural Examination

Most field isolates require serum for growth.

Inoculate BA and MCA plates and incubate at 37° C in an atmosphere containing 8 to 10 % carbon dioxide.

Serological Testing

Serological testing is used extensively for individual cow and herd diagnosis of brucellosis. Tests used include —

Serum Agglutination

Detect IgM and IgG classes. Test can be qualitative or quantitative depending on the method used. Tend to be non specific but are useful screening tests. Various serum treatments are employed to improve specificity and to help differentiate between vaccinated and naturally infected cattle. Alternatively a more specific test can be used.

Complement Fixation

Detects principally IgG1. Is a less sensitive than agglutination tests but a more reliable method for distinguishing calfhood vaccination serological responses from those due to infection with field strains.

Milk Ring Test

Is a highly sensitive but qualitative test done on vat milk to screen dairy herds for brucellosis. Antibodies in milk depend on active infection of the udder.

The antigen is a concentrated stained suspension of *B. abortus* organisms which is added to milk. In positive samples the antibodies agglutinate the antigen which rises to the surface with fat globules to form a coloured ring in the cream layer.

Herds giving a positive reaction are classified as suspect. Cows are then individually blood tested to determine the infectious status of each animal.

Brucella ovis

Microscopic examination of semen (Koster's staining method. IF)

Brucella ovis, semen smear stained with dilute carbol fuchsin.



Tiny coccobacilli can be seen (with difficulty) within PMN cells and extracellularly.

Because this smear has been stained only with dilute carbol fuchsin all structures are staining pink.

With the differential MZN or modified acid fast staining technique brucellae stain pink against a blue background. Non-modified acid fast bacteria also stain blue.

Semen culture

Serological Diagnosis

Complement fixation test.

ELISA.

Brucella ovis on sheep blood agar after 6 days in 10% CO₂ atmosphere.

