



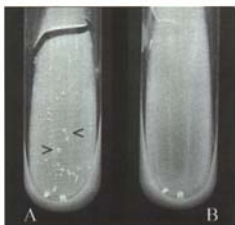
Extension Bulletin E-2734, New, December 2000

Laboratory Methods Used for the Diagnosis of Bovine TB: Culture and the Polymerase Chain Reaction

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Diagnosis of bovine TB in animals involves many steps. Initial steps involve the use of the caudal-fold tuberculin test (CFT) and the comparative cervical tuberculin test (CCT) to identify animals suspected of being infected with bovine TB. On the basis of these first tests, animals suspected of being infected with bovine TB are sacrificed and examined at necropsy for gross and histological (microscopic) lesions compatible with the disease. A definitive diagnosis of bovine TB requires identifying *Mycobacterium bovis*, the bacterium that causes bovine TB, in suspect animals. Two methods are used for identifying *Mycobacterium bovis* in infected animals: culture and the polymerase chain reaction (PCR).

Mycobacterium bovis Culture



Culture (a) positive and (b) negative for *Mycobacterium*.

to ensure the accuracy of their culture methods. Culturing for *Mycobacterium bovis* is performed under

Tissue samples collected at necropsy from cattle suspected of being infected with bovine tuberculosis (TB) are submitted to an appropriate animal diagnostic laboratory for the attempted culturing of *Mycobacterium bovis*. Because of the importance of bovine TB, these laboratories follow strict procedures

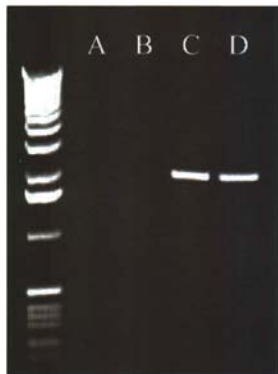
specific conditions that favor the growth of the bacteria. Unfortunately, *Mycobacterium bovis* are very slow-growing organisms and can take 8 to 16 weeks to grow. Therefore, a culture is not called negative for bovine TB until after it has been incubated for 3 to 4 months. If an organism is isolated from submitted tissue samples, it is subject to further testing to determine if the isolate is *Mycobacterium bovis* or some other closely related *Mycobacterium* species. This is done using biochemical and genetic testing. Each species of *Mycobacterium* has a specific biochemical and genetic makeup that can be identified in the laboratory. This allows for definitive classification of an isolated organism to a specific *Mycobacterium* species.

Polymerase Chain Reaction (PCR)

The polymerase chain reaction is a powerful tool that is used in a wide variety of diagnostic procedures. PCR is used to detect the presence of genetic material (DNA) that is unique and specific to the organism of interest. PCR works by amplifying a portion of DNA that is specific for the organism of interest. This product is then visualized using a laboratory procedure called agar gel electrophoresis. PCR is very sensitive and can detect the presence of an organism at very low levels. For the diagnosis of bovine TB, PCR is used to identify *Mycobacterium bovis* in tissues collected at necropsy from cattle suspected of being infected with bovine TB. PCR is used only on tissues that are histologically (microscopically) compatible with bovine TB infection. The results can typically be obtained within 1 to 2 weeks and



are classified as either positive or negative. Regardless of the results of the PCR test, tissue samples are also submitted for the attempted culture of *Mycobacterium bovis*. However, a positive test obtained on PCR is highly suggestive that the animal is infected with bovine TB and, along with other test results, may be used to classify an animal as infected with bovine TB.



DNA amplified by PCR can be visualized by agar gel electrophoresis. In this example, DNA from the organism of interest was present in sample C and D but not sample A and B. The left-hand lane is a standard size indicator.

DNA Fingerprinting

After an organism has been isolated and confirmed as *Mycobacterium bovis*, it can be further characterized to determine its genetic relationship to other isolates of *Mycobacterium bovis* using a technique called DNA fingerprinting. Briefly, well characterized specific sections of *Mycobacterium bovis* DNA are broken into pieces by enzymes. These enzymes work at specific sites along the length of the DNA and are defined by genetic sequences. After being broken into pieces, the DNA is visualized, and the number and length of the pieces create a pattern or "fingerprint." Isolates of *Mycobacterium bovis* that are closely related will have identical or very similar DNA fragment patterns. Comparing patterns from various *Mycobacterium bovis* isolates may lead to conclusions about the possible origin of newly identified isolates. DNA fingerprints from six bacteria of the same species are illustrated below. The DNA fingerprints of isolates A-D are identical; the DNA fingerprints of isolates E and F are different. It can be concluded that isolates A-D are more closely related to one another than they are to isolates E and F.



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