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A world free of bTB

The only source for all official OIE-prescribed bovine tuberculosis tests

ThermoFisher SCIENTIFIC

Diagnosis of bovine tuberculosis

Bovine tuberculosis (bTB) is a major infectious disease among cattle, other farm animals, and certain wildlife populations. It is caused by infection with mycobacteria belonging to the *Mycobacterium tuberculosis* complex, in particular *Mycobacterium bovis,* and can be transmitted either through respiration or by ingestion.

Diagnosing bTB is complicated, and a diagnostic gold standard that can detect all infected animals is not currently available. Conventional diagnostic tools (i.e., detection of antibodies or antigens) can be used only in the late stages of the disease. Consequently, the most widely used bTB diagnostics are based on the cell-mediated immune response, which is determined by either skin or blood testing.

The OIE prescribes skin testing and IFN-γ blood testing (i.e., Applied Biosystems™ BOVIGAM™ assay) for bTB diagnosis. The European Food Safety Authority has recommended the IFN-γ test for inclusion in the official list of stand-alone bTB tests for premovement testing.[1]

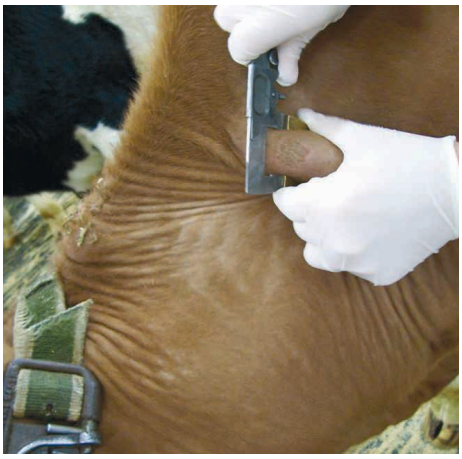
Our portfolio of Applied Biosystems™ BOVIGAM™ tuberculin purified protein derivative (PPD) kits, combined with the Applied Biosystems™ VetMAX™ *M. tuberculosis* Complex
Real-Time PCR Kit for infection confirmation, offers the only complete range of OIE-prescribed bTB diagnostic tools.

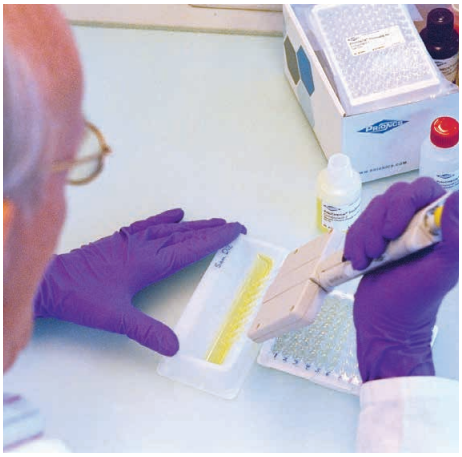
A world free of bTB

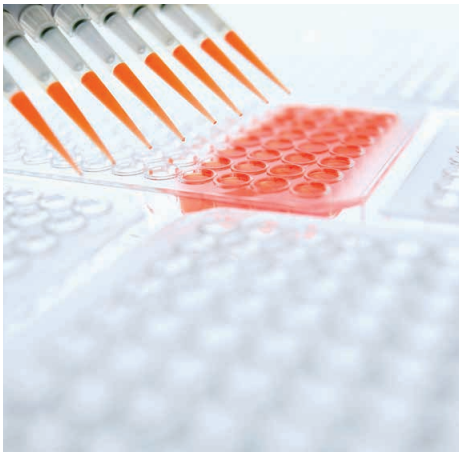
A world free of bTB may be realized when an appropriate combination of testing schemes is selected and implemented. We are working to provide the solution through our tuberculin PPD for skin testing and BOVIGAM assay for laboratory testing. These diagnostic tools can be used in various applications to help increase test sensitivity and/or specificity, according to local needs.

The flexibility in the application of bTB tests and test methods is extensive. For example, different bTB skin testing regimens such as the comparative cervical test (CCT) and the single cervical test (SCT) can be applied. Alternatively, various combinations of antigen stimulants (tuberculin PPDs and Applied Biosystems™ BOVIGAM™ PC-EC and PC-HP Stimulation Antigens), in combination with the BOVIGAM assays, can be used to help increase the specificity or sensitivity of the IFN-γ test. Moreover, the skin test and BOVIGAM assays have often been combined to help either increase specificity during serial testing or ensure that all bTB-positive animals have been identified with parallel testing. The choice of tests and their applications is dependent on both the risk of bTB infection in a region and the goal of a bTB program.

Here we explain the various ways the skin test can be combined with the BOVIGAM assay according to different bTB situations.[2] The optimal application of testing schemes can help avoid unnecessary culling and lengthy farm closures, and may eventually eliminate the occurrence of bTB worldwide.







We can help you find the best solution for your local situation.

Unique product portfolio of bTB tests

BOVIGAM assay

OIE-registered diagnostic kit for bTB in cattle, sheep, goats, and buffalo (*Syncerus caffer*) based on the detection of IFN-γ:

- Enables faster results; farm operations are back in business sooner than with skin testing
- Helps to minimize negative economic impact on the farm
- Very high analytical sensitivity
- Combinations of the BOVIGAM kit and stimulation antigens can help optimize every local bTB program
- Objective and highly reproducible
- Established technology; has been applied successfully since 1991 in bTB programs
- Only one vet visit required (for blood sampling) compared to two for skin testing

Applied Biosystems™ BOVIGAM™ 2G assay

Makes use of the reliable quality of the BOVIGAM assay, but takes advantage of M.O.R.E. benefits with the BOVIGAM 2G assay:

- **M**ore robust, with improved repeatability for borderline samples
- **O**ne-component substrate
- **R**educed cost per animal, due to flexible test capacity
- **E**asy to use, due to streamlined protocol

Stimulation antigens for the BOVIGAM assay

Optimize your local bTB program by choosing the appropriate combination of stimulants to meet your needs:

BOVIGAM Tuberculin PPDs—for higher sensitivity and equivalent high specificity; improved quality through matched potencies of bovine and avian tuberculin PPDs

BOVIGAM PC-EC Stimulation Antigen—peptide cocktail for the highest specificity

BOVIGAM PC-HP Stimulation Antigen—peptide cocktail for combined high sensitivity and specificity

Applied Biosystems™ BOVIGAM™ Pokeweed Mitogen—control for the quality of the blood sample and thus proper functioning of the test

Prionics™ Tuberculin PPD skin test reagents

- Products meet requirements as described in the European Pharmacopoeia monographs
- Unique double matching helps ensure that the actual potency of bovine PPD is higher than that of avian PPD and that their difference does not exceed 500 IU/dose
- Kit validated for use with animals starting from birth
- Reagents manufactured in one of the world's largest tuberculin PPD production sites
- May be transported at –2°C to 37°C for a period of up to 14 days for convenient and cost-efficient delivery

VetMAX *M. tuberculosis* Complex Real-Time PCR Kit

- Sensitive and specific detection of all mycobacteria belonging to the *M. tuberculosis* complex, including *M. bovis* and *M. caprae* from tissues
- Fast and simple to use
- Confirmatory test for suspicious cases

Approved by official bodies

The BOVIGAM assay is:

- Included in the OIE register of diagnostic kits[3]
- Authorized for use under EU legislation (Directive 64/432/EEC)[4]
- Recommended by the EFSA for inclusion in the list of official tests for stand-alone bTB diagnosis[1]
- Used in all major bTB programs worldwide
- Authorized for use by the Friedrich Löffler Institute (FLI), Germany[5]

Prionics Tuberculin PPD skin test reagents have been:

- Registered in several countries worldwide and have successfully passed the extensive decentralized approval procedure as laid down in EC Directive 2001/82/EC[6] in several European Union countries
- Used to manufacture the WHO world reference standard

Earliest bTB detection achieved by the BOVIGAM assay

- Differences exist among bTB tests with respect to the time point and the sensitivity of detection of the disease. The IFN-γ test (i.e., BOVIGAM assay) allows for the earliest detection, followed closely by the skin test. Serology tests for antibody response or antigen detection, and pathological examinations, can be used in later stages of the disease.

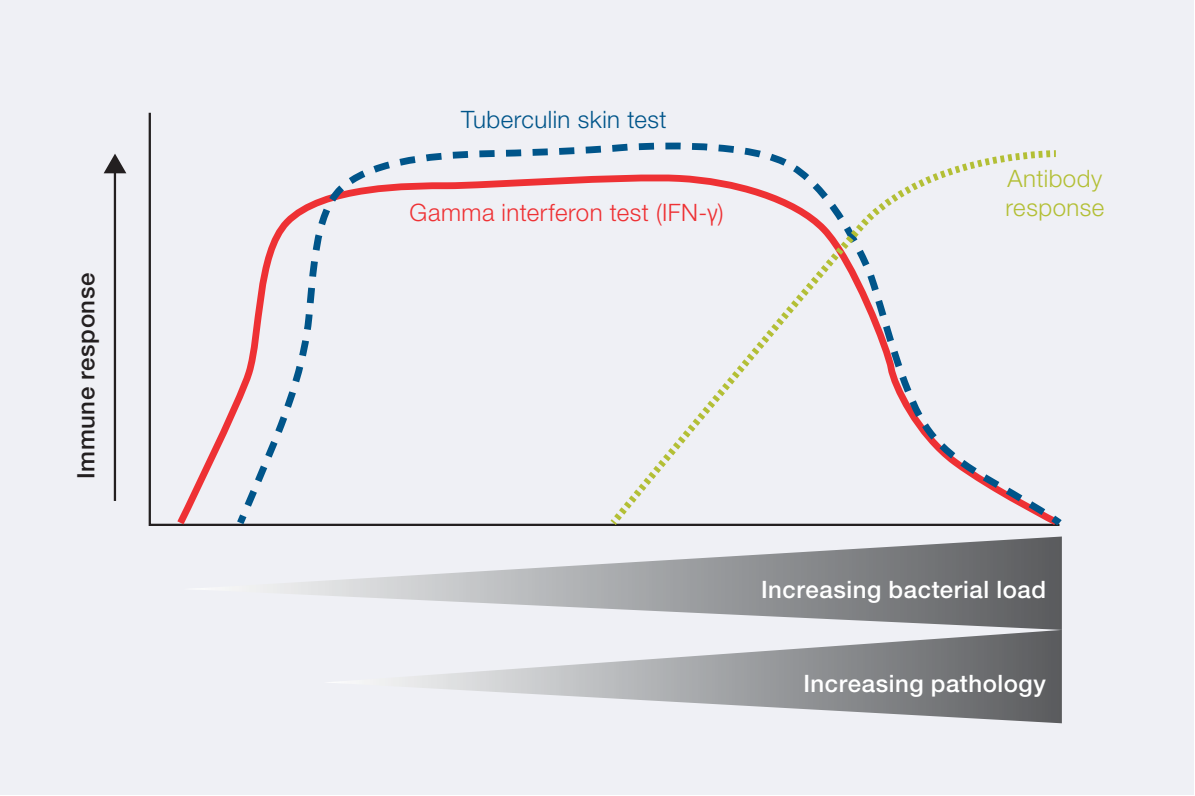


Figure 1. Time point and sensitivity of bTB detection with different diagnostic methods.
(Modified from Vordermeier M et al. 2004, *Vet Rec* 155:37–38)

Optimal results with combined use of skin tests and BOVIGAM products

Skin tests and BOVIGAM products identify overlapping, but not identical, populations of infected animals. The application of the tests in parallel enables detection of the maximum number of infected animals, whereas the application of BOVIGAM products and skin tests as serial tests helps improve specificity (Figure 2).

Use of BOVIGAM products may reduce duration of farm closures

Testing with a skin test followed by BOVIGAM products may help shorten the duration of closures for bTB-infected farms in comparison to using only skin tests. Retesting of skin test–positive animals with BOVIGAM products can be performed simultaneously with the skin test reading.

For comparison, a repeat skin test cannot be performed until at least 6 weeks after the first test. Under field circumstances (i.e., in naturally infected animals), BOVIGAM assay results are not influenced by a previous skin test (CCT).[7] With BOVIGAM products, closed herds can be retested within short time intervals, allowing the removal of newly infected animals that did not react in the earlier test. This helps prevent the silent spreading of the disease in the herd and helps to resolve confirmed bTB breakdowns more quickly.

With the combined use of skin tests and BOVIGAM products, herds can be declared free of bTB within as little as 4 days.

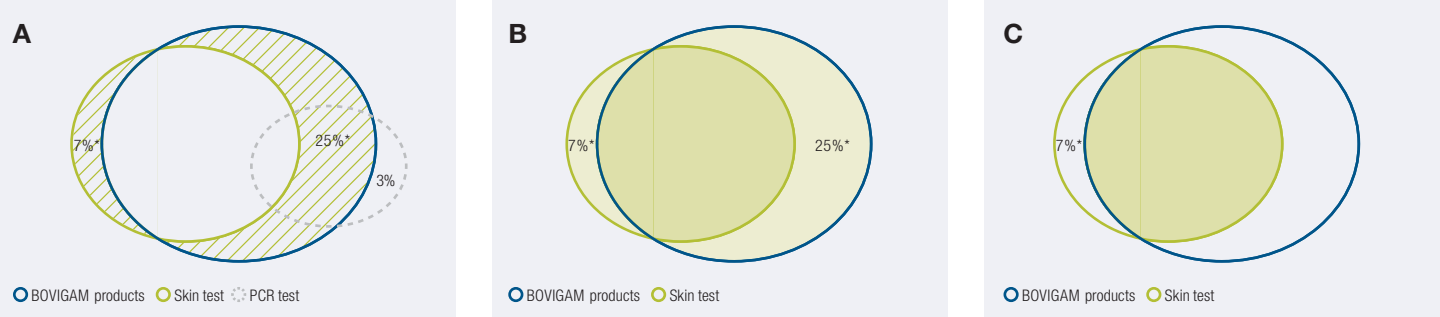
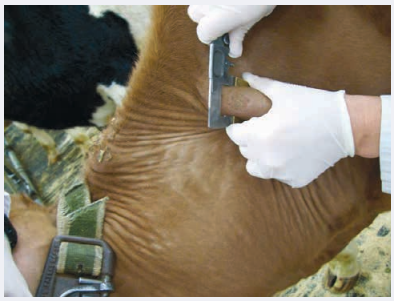


Figure 2. Combined use of BOVIGAM products and skin tests. (A) Depending on the test applied, up to 7% of reactors are detected only using the skin test (green circle), whereas up to 25% of reactors are detected only by BOVIGAM products (blue circle). The VetMAX *M. tuberculosis* Complex Real-Time PCR Kit can be used to confirm suspicious cases (dotted line). **(B)** For maximum sensitivity, animals reacting in either or both tests are removed from the herd (i.e., parallel testing). **(C)** Maximum specificity is obtained when only the reactors from both tests are removed from the herd (i.e., serial testing). * Depending on skin test applied.

Unique doubly matched skin test reagents

Our tuberculin PPDs meet the requirements described in the European Pharmacopoeia (EP) monographs [0536] and [0535]. The Tuberculin PPD Kit contains avian (2,500 IU/dose) and bovine (3,000 IU/dose) tuberculin PPD matched for the SICCT test. The potencies of the Prionics Bovine and Avian Tuberculin PPDs are doubly matched during their production. This means that the actual potency of bovine tuberculin PPD kits is higher than that of avian tuberculin PPD, and that the difference in potency between the two tuberculin PPDs does not exceed 500 IU/dose. Matching of the potency of tuberculin PPDs to each other is not required in the EP monographs. Combining tuberculin PPDs in a skin test that are merely matched based on their stated potency is relatively imprecise because the EP monograph allows a large deviation between the stated and the actual potency.



Unique double matching ensures that the actual potency of bovine tuberculin PPD is higher than that of avian PPD and that their difference does not exceed 500 IU/dose.

Risk-based test schemes

Based on the suspected bTB incidence in a herd, different testing schemes should be applied.[2]

Figure 3. Herds with varying bTB incidence.

Infected herd

Status: High risk of high bTB prevalence in a herd.
Goal: Detection of all positive animals in a bTB outbreak herd.
Testing scheme: All animals are subjected to an SCT or CCT skin test. CCT is used mainly if the level of environmental exposure to the other sensitizing organisms is high or unknown. All skin test–negative animals are retested with BOVIGAM products (stimulation with tuberculin PPD and/or BOVIGAM PC-HP Stimulation Antigen).
Action: All skin test and BOVIGAM reactors are removed from the herd (see Figure 2B).

Herd with points of contact with infected herd

Status: Moderate risk of high bTB prevalence in a herd.
Goal: Test with high specificity in order to identify all infected animals without producing false-positive results.
Testing scheme: All animals are subjected to a skin test (CCT). All positive-testing animals are retested with BOVIGAM products (stimulation with tuberculin PPD and BOVIGAM PC-HP or PC-EC).
Action: Options differ depending on local requirements. Either animals positive in both tests are removed from the herd (high specificity; see Figure 2C), or animals positive in either CCT or BOVIGAM assays are removed from the herd (high sensitivity; see Figure 2B).

Neighboring herd

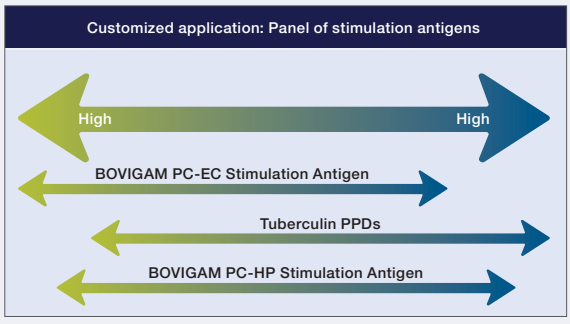
Status: Low risk of high bTB prevalence, or absence of infection.
Goal: Test with high specificity to avoid the detection of false-positive animals and with high sensitivity to detect positive herds with the greatest probability.
Testing scheme: All animals are subjected to a skin test (CCT) to identify positive herds. All CCT-positive animals are retested with BOVIGAM products (stimulation with BOVIGAM PC-EC Stimulation Antigen or confirmed by PCR).
Action: Only animals positive in both tests are removed from the herd (see Figure 2C).

Some animals may be inconclusive in the skin test. Such animals are either positive or negative in the BOVIGAM test. The subsequent action depends on the bTB prevalence in a herd and on the local situation.[2]

The correct application of testing schemes avoids unnecessary culling and lengthy farm closures, and is a good step toward a world free of bTB.

Versatile and effective BOVIGAM IFN-γ tests

BOVIGAM and BOVIGAM 2G assays are the only blood-based assays for cell-mediated immunity currently on the market that have been validated together with tuberculin PPDs and a panel of peptide stimulants. The combined use of these different peptide stimulants with tuberculin PPDs allows adjustment of the application of the test to specific TB situations.



Tuberculin PPDs in combination with the BOVIGAM assays offer the highest sensitivity, BOVIGAM PC-EC Stimulation Antigen offers the highest specificity, and BOVIGAM PC-HP Stimulation Antigen combines high sensitivity with high specificity.

Combinations of BOVIGAM products and stimulation antigens can help optimize every local bTB program.

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Ordering information

Product	Quantity	Cat. No.
Interferon-γ release assays		
BOVIGAM 2G TB Kit	10-plate kit (300 samples)	63330
BOVIGAM TB Kit	10-plate kit (150 samples)	63320
BOVIGAM TB Kit	30-plate kit (450 samples)	63326
Mycobacterium bovis Gamma Interferon Test System für Rinder BOVIGAM	10-plate kit (150 samples); incl. 2 x PPD and 1 x PWM	7610910 (Germany)
Stimulation antigens		
BOVIGAM Tuberculin PPD		
Bovine Tuberculin PPD 3000	5 mL; 30,000 IU/mL	7600060
Avian Tuberculin PPD 2500	5 mL; 25,000 IU/mL	7600065
Peptide cocktails		
BOVIGAM PC-EC Stimulation Antigen	3 mL (lyophilized); 120 samples	7600100
BOVIGAM PC-HP Stimulation Antigen	3 mL (lyophilized); 120 samples	7600105
Stimulation control		
BOVIGAM Pokeweed Mitogen (PWM)	1 vial; 3.2 mg	5108777
PCR		
VetMAX *M. tuberculosis* Complex Real-Time PCR Kit	100 reactions	MTBC
Tuberculin PPD for skin test*		
Tuberculin PPD Kit (contains avian (2,500 IU/dose) and bovine (3,000 IU/dose) tuberculin PPD; matched products suitable for SCT and SICCT)	20 x 2 mL, 20 x 20 doses each	A49422
Tuberculin PPD Kit (contains avian (2,500 IU/dose) and bovine (3,000 IU/dose) tuberculin PPD; matched products suitable for SCT and SICCT)	10 x 5 mL, 10 x 50 doses each	A49425
Bovine Tuberculin PPD 3000	40 x 2 mL, 800 doses	A49396
Bovine Tuberculin PPD 3000	20 x 5 mL, 1,000 doses	A49400
Avian Tuberculin PPD 2500	40 x 2 mL, 800 doses	A49432
Avian Tuberculin PPD 2500	20 x 5 mL, 1,000 doses	A49414

* These are regulated products and require a special order process. For more information, email Prionics Lelystad BV at **orders.prionics@thermofisher.com**

References

1. Scientific opinion on the use of a gamma interferon test for the diagnosis of Bovine Tuberculosis. *EFSA Journal* 2012;10(12):2975.
2. National guidelines apply.
3. Resolution No. 34, Register of Diagnostic Kits Validated and Certified by the OIE (Registration Number: 20150110).
4. Council Directive 64/432/EEC on animal health problems affecting intra-community trade in bovine animals and swine.
5. Authorization number FLI B-484.
6. EC Directive 2001/82/EC on the community code relating to veterinary medicinal products.
7. Schiller I et al. (2010) Bovine Tuberculosis: effect of the tuberculin skin test on *in vitro* interferon gamma responses. *Vet Immunol Immunopathol.* 136(1-2):1–11.

Find out more at **thermofisher.com/animalhealth**

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