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Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Bovine Tuberculosis

Manual

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Introduction

The Bovine Tuberculosis Scheme was officially introduced by the Division of Veterinary Services during 1969 with the purpose of eradicating the disease in the Republic of South Africa.

At the initial stage it was already realized that the need for testing for tuberculosis (TB) in herds varied and that it was largely determined by the type of farming being conducted. In order to incorporate as many stock owners in the scheme as possible, the testing procedures and the various testing programmes were adapted through the years. Such modifications also necessitated changes in the administrative processes and consequent adaptations to the manual.

The various testing programmes as set out in the manual should be regarded as appropriate means of achieving the final objective of the programmes: the total eradication of TB. In order to reach this goal, thorough planning and careful use of available funds and manpower would be required. Extensive knowledge of the scheme as presented in this manual is a prerequisite for sensible planning and execution of the programme.

The scheme begins with comprehensive guidance to stock owners who wish to join and for his herd to be assigned to the correct testing programme. The previous accreditation programme has been discontinued and replaced by the present scheme. Thus, stud and dairy herds should be incorporated into the Maintenance (old Annual Diagnostic) Programme as many of these require a declaration for the sale of stud animals or milk. Where there is no need for a TB -free declaration or where such a declaration is not of much value to the owner, the incorporation should be into the Surveillance (old Herd Diagnostic) Programme and *not* the Maintenance Programme. A single negative herd test under the Surveillance Programme makes it possible to test many more herds to track down bovine TB and thereby achieving the final objective sooner.

The Bovine Brucellosis Eradication Scheme, launched by the Division of Veterinary Services during 1978 primarily has the same final objective, namely the eradication of brucellosis in cattle in South Africa. The Bovine Brucellosis Eradication Scheme is conducted in much the same way as the TB Eradication Scheme and the possible synchronizing of tests within the same herd under the two testing schemes should be borne in mind.

1 Tuberculosis - The Disease

Definition: Tuberculosis (TB) is a chronic disease caused by infection with a member of the *Mycobacterium tuberculosis* complex which comprises *M. tuberculosis*, *Mycobacterium bovis* (incl. BCG and *Mycobacterium caprae*), *Mycobacterium microti*, *Mycobacterium africanum*, *Mycobacterium canettii* and *Mycobacterium pinnipedii*. All mycobacteria are acid fast and those belonging to the *M. tuberculosis* complex can affect the majority of vertebrates.

Three types of TB are important, namely bovine TB (BTB) caused by *M. bovis*, avian TB caused by *Mycobacterium avium*, and human TB caused by *M. tuberculosis*. Humans may also develop TB due to *M. bovis* as it is a zoonotic pathogen. Although these diseases are related, the causative organism differs in all three cases and can be identified by laboratory tests. The organisms can also cause the disease in animals other than cattle, birds and humans. Avian tuberculosis (caused by *M. avium*) is rather referred to as a mycobacteriosis and not discussed fully in this document.

1.1 Susceptibility of animals to the three *Mycobacterium* strains

Animal	<i>M. bovis</i>	<i>M. avium</i>	<i>M. tuberculosis</i>
Cattle	XXX	X	X
Fowl	-	XXX	
Human	XXX	XX	XXX
Pig	XXX	XX '	XX '
Sheep	XX '	XX '	XX
Goat	XXX	XX	XX
Horse	XX '	XX	XX
Cat	XXX	XX	
Dog	XX '	XX	XXX

Meaning of symbols:

XXX	susceptible - visible lesions develop
XX '	susceptible - visible lesions sometimes develop
XX	susceptible - visible lesions seldom develop
X	visible lesions usually do not develop but animals react to the tuberculin test

To predict the susceptibility of wild animals to different strains of mycobacteria is nearly impossible, mainly due to the fact that the susceptibility is most likely influenced by several aspects inherent to both the host and the pathogen, and very likely the environment as well – see wildlife section for more detail.

1.2 History

TB has been known since classical times when it occurred in Europe and Great Britain. In South Africa the disease was recorded for the first time in 1880. It is apparent that TB was introduced into the local cattle population by the introduction of European breeds of cattle. In 1902 Natal was reported to be free of TB but TB was found in a large numbers of imported animals. These animals were destroyed. However, the disease still spread through the country, mainly in dairy herds, but, as farming systems became more intensive, the disease was commonly found in beef herds as well. Due to the increasing prevalence, TB was declared a

1.3 Pathogenesis of the disease

1.4 Transmission

Infection through the mouth also occurs via infected milk or where the food, water, grazing (especially irrigated pastures) or mineral and feed licks are contaminated by bacteria from open lung lesions where mucus is coughed up. Water and feed troughs may therefore be an extremely important source of infection. It can also be transmitted where the saliva or food in the alimentary canal is infected by lesions in the canal itself (the organism may then be excreted in the faeces). The organism may also be excreted in the milk, urine and vaginal fluids if these organs are infected. Infected lymph nodes that erupt (burst) on the skin may also contaminate the environment, food and water sources as mentioned.

The period that the bacteria remain infectious outside the body depends on climatic conditions. Desiccation and direct sunlight are detrimental to the organism and will reduce the length of time that it remains infectious. The organism can remain infectious in stagnant water for up to 18 days while it can remain viable in moist soil for up to 8 weeks.

Humans most commonly become infected with *M. bovis* by drinking unpasteurised infected milk.

1.5 Symptoms

The vast majority of infected cattle that react positively to the tuberculin test show no obvious clinical symptoms. Animals with diffuse lesions throughout the body may later gradually become emaciated, lethargic and anorexic with a fluctuating temperature and a dull coat. These symptoms become evident during stress and when the body is subjected to physical exertion such as during calving. These types of symptoms take many months to develop.

With advanced infection of the lungs, a single suppressed moist cough may occur especially early in the morning after exercise, when it is cold or in dusty conditions. Shortness of breath is apparent in advanced cases where excessive lung tissue is diseased. Enlarged bronchial lymph nodes may exert pressure on the airways and this can lead to dyspnoea (difficulty in breathing). Continuous bloat can occur as a result of pressure on the esophagus through enlarged mediastinal lymph nodes.

If the lymph nodes such as the sub-maxillary, pre-scapular, pre-crural and supra-mammary nodes are infected, they may sometimes be seen or felt on palpation.

Tuberculous metritis (uterine infection) can hamper conception or it can result in abortion in advanced pregnancy.

Infection of the udder is a source of transmission of TB organisms to the calf or people who drink the infected milk (if it is not boiled or pasteurized). It is sometimes difficult to distinguish this type of mastitis from mastitis caused by other organisms. In cases of TB mastitis one may typically see hardening and/ or enlargement of the top part of the udder hind-quarters. In such cases the supra-mammary lymph nodes become enlarged. If these lymph nodes become enlarged without palpable lesions in the udder itself, it may potentially point to TB infection. Usually the milk initially looks normal in TB mastitis cases. Later, flakes may become visible if the milk is left standing for a while and in advanced cases the milk (excretion) becomes a transparent light yellow-brown colour.

1.6 Diagnosis

1.6.1 Field tests

a.) Intradermal tuberculin test

This is the definitive **screening test** that is currently used. It is the most commonly used test and most practical test. It is also an OIE (World Organisation of Animal Health) prescribed test for international trade.

Tuberculin is a solution of protein material extracted from the cell wall of the *Mycobacteria* organism. When this protein solution is injected into the skin of an infected animal, the body's sensitised immune response will cause a localised inflammatory reaction that leads to the typical signs of a positive tuberculin test. Animals that have not been exposed to tuberculosis will not mount an inflammatory reaction (see Chapter 2 Immunology).

The preferential site for conducting this test is the neck area (see Chapter 3). A caudal fold test using tuberculin has been used in the past but is not currently used in South Africa.

The various different types of Mycobacteria all have cell membranes containing closely related chemical compounds. It is therefore not surprising that the various types of Mycobacteria cause a degree of sensitivity for mammalian tuberculin. Fortunately, in the case of such a cross reaction, the reaction to mammalian tuberculin is less severe than the reaction to a tuberculin of the Mycobacterium concerned. This enables us to determine the sensitivity caused by *M. avium* or related Mycobacteria by means of the comparative test where mammalian and avian tuberculin are used simultaneously.

The Stormont, double intradermal, caudal fold and temperature tests (where tuberculin is used) have been used in the past, but are no longer used as they have no compelling advantage over the cervical intradermal tuberculin test.

1.6.2 Laboratory Diagnosis

a.) Direct Examination

Smears can be made from: Bits of mucus that have been coughed up; the sediment after milk has been centrifuged; lymph nodes; or other excretions and organs. These smears are then stained according to the Ziehl- Neelsen method and examined under the microscope.

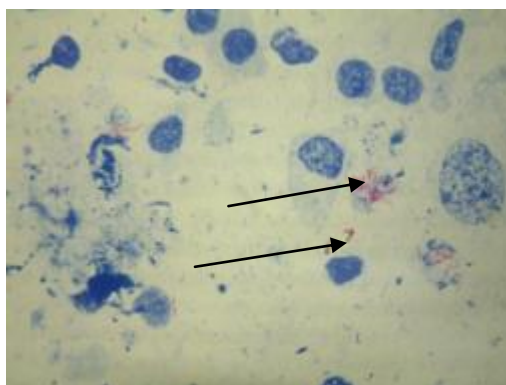


Figure 1 Acid-fast organism

According to the OIE manual, the fluorescent acid fast and the immunoperoxidase methods could also be used for direct microscopic examination.

b.) TB Guinea pig inoculation test (Biological Test)

Suspensions of the tissue samples from suspected infected cases can be injected into guinea pigs and then a post mortem is conducted 6 weeks later to identify typical TB lesions if the organism was in fact present.

c.) Culture Test

This is the definitive **diagnostic test** and should be used for all suspect and positive herds. The same type of material that is used in the previous two tests is inoculated onto special media and cultured. This is a time-consuming test as Mycobacteria are slow growing and it may take six to eight weeks before a diagnosis can be made.

d.) Gamma Interferon Test

This is an in vitro test for cellular immunity. It is a useful auxiliary test when used in combination with the intradermal tuberculin test as it increases the sensitivity by up to 20% (sensitivity = number of positive animals that test positive). This test cannot be used in isolation to make a diagnosis. Blood samples have to be processed rapidly (within 2-6 hours) after collection to ensure that the immune system cells still remain viable for the testing procedure.

e.) ELISA

The ELISA serological test measures humoral antibodies which develop during the course of the disease. The ELISA test can also be used to detect specific Mycobacterial antigens. According to the OIE Manual, the ELISA could also be useful for detecting *M. bovis* in wildlife. Anergic reactors may potentially be picked by the ELISA up as humoral immunity (antibodies) increases over time as cellular immunity decreases.

f.) Flourescence Polarization Test

This is a newly developed test which is still under investigation.

g.) PCR

This test utilizes the polymerase chain reaction to detect target DNA in tissue and other specimens. Specific DNA primers are used to hybridize with target DNA in specimens. The test requires

thermocycler equipment which is expensive.

1.6.3 Post Mortem

In performing a post mortem examination for TB, attention is mainly focused on the lymph nodes as they are usually infected if the organ (or the part of the body) from which they drain lymph is infected. The expected degree of infection of the lymph nodes and organs are given in descending order:

Lymph node / Organ	Expected degree of infection
Bronchial, Mediastinal or Mesenteric Lymph nodes	90%
Lungs	75%
Pleura	55%
Peritoneum	50%
Liver	50%
Spleen	50%
Pericardium, Uterus and kidneys	10%
Trachea	5%
Udder and ovaries	0.5 - 1%

When the lymph nodes are infected, tubercles are formed that enlarge and later coalesce, eventually becoming caseous and calcified.

In buffalo it has been found that the lesions can be extremely small and the cut surfaces of the lymph node must be carefully examined in good light.

When doing a post mortem examination, all lymph nodes of the head, neck and thoracic cavity must be carefully examined and numerous cuts made into the lymph node tissue. Whenever signs of systemic TB are seen, lymph nodes in all common sites in the body must be examined as well.



Figure 2 - Tuberculosis in lymph node (Buffalo)

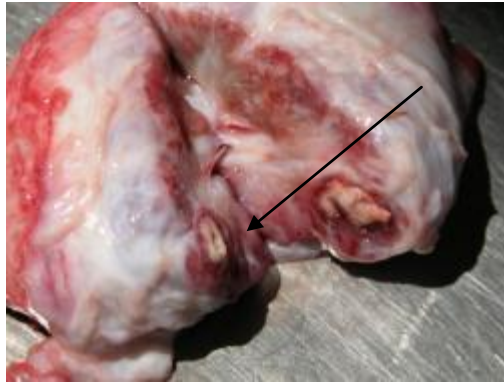


Figure 3 - Discrete lesion in lymph node

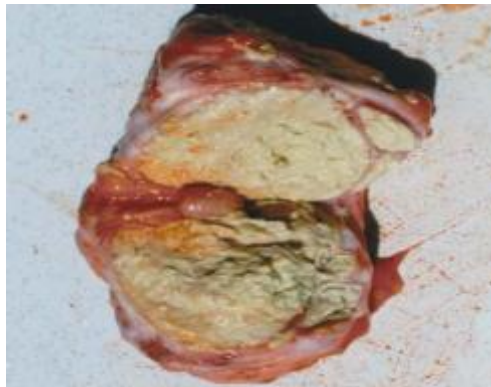


Figure 4 - Lymph node showing caseation

In the lungs of infected cattle, large nodules are seen with normal lung tissue between them. These nodules increase in size and often coalesce to form large areas where the normal lung tissue is completely destroyed. When these nodules are cut open, they usually have a caseous centre and older lesions are often 'gritty' due to the calcification.



Figure 5 - Tuberculosis in lungs

Note these lesions may also be very small and only identified if the lung tissue is carefully palpated. Chronic lesions in the lung are often surrounded by a thick fibrous capsule.



Figure 6 - Tuberculosis in lungs

In cattle, tuberculous pleuritis and peritonitis are often present. This condition can occur without the lungs, liver, spleen or kidneys being infected – this occurs when infection has taken place through the lymphatic ducts. Granular TB nodules that form on the pleura consolidate to form clusters almost like a bunch of grapes. These lesions also have caseous and sometimes calcified content surrounded by connective tissue. Similar lesions are found on the peritoneum. Adhesions to other organs may also be present.

Organs such as the liver, spleen, kidneys and the reproductive system of both sexes must also be examined. Infection of the bones, especially the ribs, vertebrae and joints must not be overlooked.

In the case of infection of the udder it is sometimes found that only one quarter (especially a hind-quarter near the attachment of the udder) is involved. In chronic cases the lesion develops slowly, with hardening and enlargement of that quarter, without pain being elicited. The normal grey-white elastic udder tissue changes and becomes yellowish brown with a firm consistency.

Samples for laboratory examination

Under extensive farming conditions TB usually spreads slowly. However, currently the tendency is to farm intensively with a higher stocking density. This often occurs on cultivated pastures, in feedlots and in dairies around the larger cities. Thus favourable conditions are created for the spreading of the disease in cow sheds, at feeding troughs, at water points, through licks provided and through concentrated grazing systems.

As humans are also susceptible to bovine TB, we have a duty in respect of public health to control and eradicate the disease. In a nationwide study in the USA, it has been shown that patients of Hispanic origin aged 15 years or less that were HIV positive, were more likely to be infected with *M. bovis* than by *M. tuberculosis*. Person-to-person transmission of bovine TB has also been reported. In herds where the disease is prevalent the danger exists that people may inhale contaminated air where they come in close contact with the cattle such as in cow sheds. The drinking of un-boiled or un-pasteurized contaminated milk is possibly the greatest danger for infecting people. A few cases are also known to have occurred where people have become infected through wounds (e.g. knife wounds during a post mortem examination on an infected animal).

1.8.1 Humans

All staff working on infected farms should be tested by the Department of Health.

Pigs are susceptible to infection with *M. bovis*, *M. tuberculosis*, *M. avium* and *M. avium-related* bacilli. The lesions that develop are usually not of a progressive nature. The lesions tend to diminish in size over time and to disappear or to become inactive. Where pigs become infected with *M. bovis*, it is usually through the intake of contaminated dairy products, especially milk. The disease does not have the tendency to spread from one pig to another and it usually disappears if the source of contamination, such as contaminated milk, is removed.

Pigs can be tested for TB using the intradermal tuberculin test done in the soft skin at the base of the ear. The test is read after 24 or 48 hours. In pigs with active lesions a skin reaction of 5 mm and more may be observed - sometimes even with necrosis. The test on pigs is, however, not reliable because the animals often overcome active infection and remain tuberculin skin test positive. Where pigs are the source of re-infection for cattle, measures will have to be taken to separate the pigs and their products totally from the cattle on the farm. The possible transmission of infection by workers will also have to be prevented. Slaughtering of the

pigs with proper meat inspection, if feasible, will possibly be the best solution.

1.8.3 Poultry

Poultry and birds can become infected with *Mycobacterium avium*. If there is contact between infected poultry or birds and cattle, the cattle can become infected. In cattle *M. avium* usually does not cause visible lesions, but small non-progressive lesions can appear especially in the mesenteric lymph nodes. This is because the main route of infection is oral through the contamination of cattle feed by infected poultry. In exceptional cases *M. avium* causes metritis and mastitis. Lesions in the lungs and generalized TB-like lesions may also occasionally be seen. Infection with *M. avium* sensitizes the cattle and they will react to the intradermal tuberculin test. When a comparative Intradermal test is done, these animals show a stronger reaction to the avian than to bovine tuberculin. In order to determine the source of infection post mortem examinations can be done on suspect poultry or they can be tested with avian tuberculin in their combs. If poultry are suspected of infecting cattle, all in contact poultry should be slaughtered. Further sensitizing of cattle can be prevented by preventing cattle from coming into contact with poultry or their products (such as the feeding of chicken manure to cattle) provided the chickens are the only *M. avium* source of contamination.

1.8.4 Sheep and horses

These animals have a natural resistance and are seldom infected. It therefore only happens by means of exception that the cause of re-infection of cattle will be found among these animals if all the other causes have been eliminated.

1.8.5 Goats

Goats are not infected often. However, the natural resistance of goats is low and where they are exposed to a high degree of infection with *M. bovis*, large numbers can become infected. Broncho-pneumonia can develop and the gastro-intestinal lymph nodes and other lymph nodes can be infected. Where infection in cattle is determined, it will be necessary to pay attention to goats especially if there is close contact between them and cattle. Where possible a post mortem examination can be undertaken and the tuberculin test applied if the hair is clipped short. *M. avium* infection has also been found among goats. The infection causes disease that develops slowly in goats but may be the cause for the disease spreading to other animals.

1.8.6 Pets

The possibility that dogs and cats can be a source of infection to cattle is very low. Dogs, which are readily susceptible to *M. tuberculosis* infection, are possibly a greater danger to humans than to cattle. Cats, however, are readily susceptible to *M. bovis* infection. On many farms it is customary for cats to be kept at cow sheds to kill off rodents. Usually unboiled milk is given to cats and if the milk is contaminated the cats can become infected and later become a source of reinfection to cattle.

1.8.7 Wildlife

Distribution and history (free-ranging wildlife)

Cases of TB in wildlife have been reported in different parts of the world. In 1919, cases in bison and elk in Canada were diagnosed. Of late, there are cases of badgers in England and Ireland. Cases in yellow-necked mouse, wood mouse and polecat have been reported in the UK in 2004.

In Africa, Uganda reported cases in buffalo and warthogs in 1963. A case in a baboon was reported in Kenya in 1987.

The following table summarizes cases of TB in wildlife in South Africa:

PROVINCE/AREA	SPECIES AFFECTED
Mpumalanga	Buffalo, baboon, kudu, warthog, eland, bushpig, giraffe
Eastern Cape	Kudu, duiker



Figure 7 - TB in parotid lymph nodes of Kudu



Figure 8 - Lion with TB.

Susceptibility of wildlife species:

To predict the susceptibility of wild animals to different strains of mycobacteria is nearly impossible, mainly due to the fact that the susceptibility is most likely influenced by several aspects inherent to both the host and the pathogen, and very likely the environment as well:

- 1) *Mycobacterial spp* have a very **wide host range**, and it is generally accepted that all mammals are susceptible to infection with various *Mycobacterial spp*. Almost any kind of mycobacteria can be cultured from any part of just about every wild animal. Example: baboons from KNP – although infected with *M.bovis*, the following mycobacteria were also cultured from their lymph nodes: Unknown mycobacterium of the *M. avium* complex, *M. palustre*, *M. marseillense*, *M. vulneris*, *M. abscessus*, *M.chelonae*, *M. conceptionense*, *M. sherrisii* to name but a few. It has also been speculated that the perissodactyles might be more resistant to infection with Bovine TB than the majority of the cloven hoofed species.
- 2) Susceptibility to infection and lesion morphology in various species may be related to their dominant immune pathway (CD4+, CD8+ or $\lambda\delta$ T-cells) as well as their general **immunocompetence and the infective dose of the organism**. Example: several deer species seem to be more susceptible to Bovine TB (BTB) than certain other cloven hoofed species. A study done at Agresearch, Wallaceville in NZ indicated that red deer (*Cervus elaphus*) could develop BTB lesions when infected with as few as 40 cfu *M. bovis* (Griffin et al, 2002). In a BTB infection study on African buffalo (*Syncerus caffer*) on the other hand, we proved that 3×10^2 cfu only resulted in lesions in 50% of the infected individuals, while the group infected with 3×10^4 cfu *M. bovis*, had a 100% lesion rate (De Klerk et al, 2006). Similarly there were also studies done on other species, inclusive of ferrets, stoats and weasels – which indicated that ferrets were by far the mustelid species with the highest incidence of BTB (de Lisle et al 2008) during a wildlife disease monitoring program.
- 3) Not only intrinsic factors, but also **inter / intra species interactions** as well as **behavioural** and feeding traits should be considered. Example: Animals that are more socially gregarious (buffalo, kudu, impala, lechwe) are more likely to be exposed to other infected / diseased individuals. Burrow dwelling animals (warthog, bush pig, meerkat (Parsons et al 2013), mongoose) or animals

- 4) **Duration of survival** of *Mycobacterial spp* in the environment is dependent on temperature, moisture, direct sunlight and the presence of an organic matrix. Example: In the scorching bushveld sunlight during the summer months, *Mycobacterial spp* are unlikely to remain alive for more than a couple of hours. In cooler months and in shady areas where a lot of moisture is present the survival might be extended to about 6 weeks (Tanner & Michel) whereas in Ireland / England the organisms may remain alive in the environment for up to 6 months.
- 5) The **virulence** of any specific strain of these *Mycobacteria* should also be considered. This has been a concern during several BTB discussions because “effectiveness” of BCG and plasmid vaccines has been evaluated, but virulence from different geographical areas might not have been taken into account at the time.

The reason for the difficulty of predicting susceptibility of various wildlife species is the fact that apart from buffalo and baboons (laboratory animals), no challenge studies have ever been done on other wildlife species in southern Africa. The extent to which a certain species is susceptible to a specific strain of *Mycobacteria* can only be determined if several animals of similar age and health status are challenged with different infective doses of the organisms and the outcome is measured through a panel of ante-mortem tests, necropsy, histopathology and culture.

An estimate at this stage would be that baboons are probably the most susceptible, purely because of the severity of disease progression during a short period of time, but generally disease transmission is low because of behavior. Some people might speculate that lions / leopards would be more susceptible, but disease severity and progression might be due to repeated exposure due to feeding behavior.

Bushpigs might be more susceptible than warthogs, because disease progression seem to be somewhat accelerated, but this might also be due to behavior differences (bushpigs might scavenge more than warthogs?). Bushpigs produce more offspring – thus more stressed, higher interaction rates, etc. Meerkats seem to be more susceptible than mongooses, because several meerkats have

But, from all the above speculation, the only conclusion remains the following: No susceptibility index can be compiled for wild animals if infection models with similar comparative infective doses are not established, screening tests developed and findings published on the outcomes of such models.

B-Lymphocytes (B-Cells):

These cells mediate humoral immunity. The major function of B lymphocytes is the production of antibodies in response to foreign protein (antigen) of bacteria, viruses or tumour cells. Antibodies are specialized proteins that specifically recognize and bind to one particular antigen, usually a protein, polysaccharide or lipopolysaccharide.

For every foreign antigen, there are antibody molecules specifically designed for that antigen. Antibody production and binding to the foreign substance or antigen is critical as a means of signaling other cells to engulf, kill or remove that substance from the body. There are five major classes of antibodies or immunoglobulins (Ig): IgG, IgA, IgM, IgE and IgD.

T-Lymphocytes (T-Cells):

These cells mediate cell-mediated immunity. T-lymphocytes do not produce antibodies. The specialized roles of T-cells are to directly attack foreign antigens such as viruses, fungi or transplanted tissues and to act as a regulator of the immune system.

Macrophages:

They are often referred to as scavengers because they pick up and ingest foreign materials (antigens) and present these antigens to other cells of the immune system. This is one of the important first steps in the initiation of the immune response.

Dendritic Cells:

They also capture and present antigens to cells of the immune system. They are mainly, but not exclusively, found in the structural compartment of the lymphoid organs such as the thymus, lymph nodes and spleen.

Granulocytes (Polymorphonuclear Leukocytes):

These include neutrophils, eosinophils and basophils, based on their staining characteristics with certain dyes. They are predominantly important in the removal of bacteria and parasites from the body, which they do by engulfing them, and degrading them, using powerful enzymes. They may also be effector cells in antibody-mediated auto-immune or hypersensitivity reactions, causing host tissue damage.

2.5 The Immune Response

(See also accompanying diagrams)

An immune response to a foreign antigen requires the presence of an Antigen-Presenting Cell (APC) – usually a macrophage or dendritic cell which engulfs the antigen, processes it internally and then displays parts of the antigen on its surface, thereby “presenting” the antigen to either a B-lymphocyte or T-lymphocyte.

- Cell Mediated Immunity (TB)

Helper and Killer T-cells are activated (sensitized) and multiply. Killer T-cells, with the help of the Helper T-cells and other cells such as macrophages, kill any host cell infected with that specific kind of antigen. During the replication of the T-cells, Memory T-cells are produced. These circulate in the body and result in an improved response rate to any subsequent infection by the same kind of antigen.

- Humoral Immunity (BR) (Immunity conferred by antibodies directed against a) bacterial capsules that are antiphagocytic e.g. polysaccharide capsules of *Streptococcus pneumoniae* and *Neisseria meningitidis* groups A and C in humans or to the polypeptide capsule of *Bacillus anthracis*; b) antibodies to bacterial toxins e.g. *Clostridium tetani* and *Corynebacterium diphtheriae*; and c) antibodies to viruses [viral neutralization])

B-cells are activated and multiply to form plasma cells and memory cells. The plasma cells produce highly specific antibodies (also called immunoglobulins) which inactivate the antigen using a variety of tactics. The memory cells circulate and give rise to a faster and bigger response should that specific antigen invade the body again.

Tuberculosis hypersensitivity/anergy

(see Chapter 3 for more detail)

The largest and most typical reactions to an intradermal tuberculin test occur in the early stages of infection with *M. bovis*. This sensitivity lessens later on during the chronic (advanced) phase of the disease when the animal's immune system gradually switches from a cell-mediated reaction (responsible for the skin reaction) to a humoral antibody – based reaction. Thus the intradermal test becomes less effective in detecting infected animals and in some cases the sensitivity disappears completely and the animal, although still infected, shows no reaction to the tuberculin test at all. This is known as anergy.

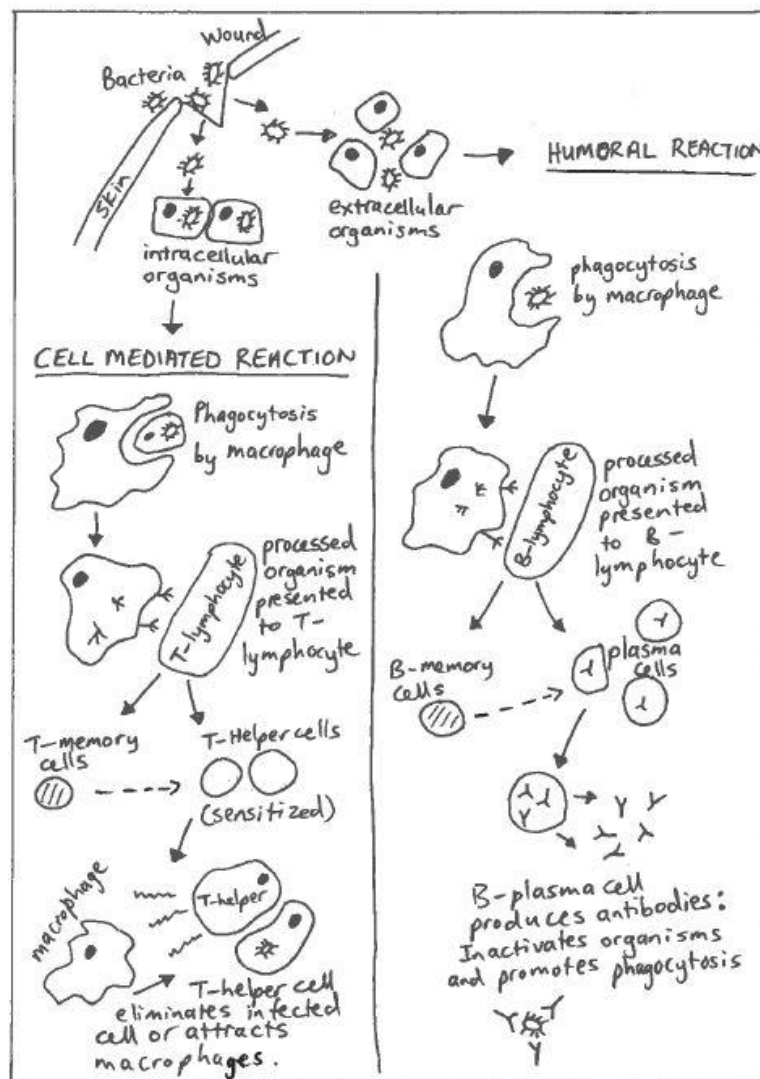
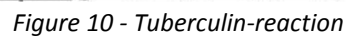


Figure 9 - The immune reaction (cell-mediated and humoral)



12 h: Lymphocytes start to migrate from the local blood vessels.

72 h: Congregation of cells result in the thickening of the skin. This is a visible and measurable reaction.

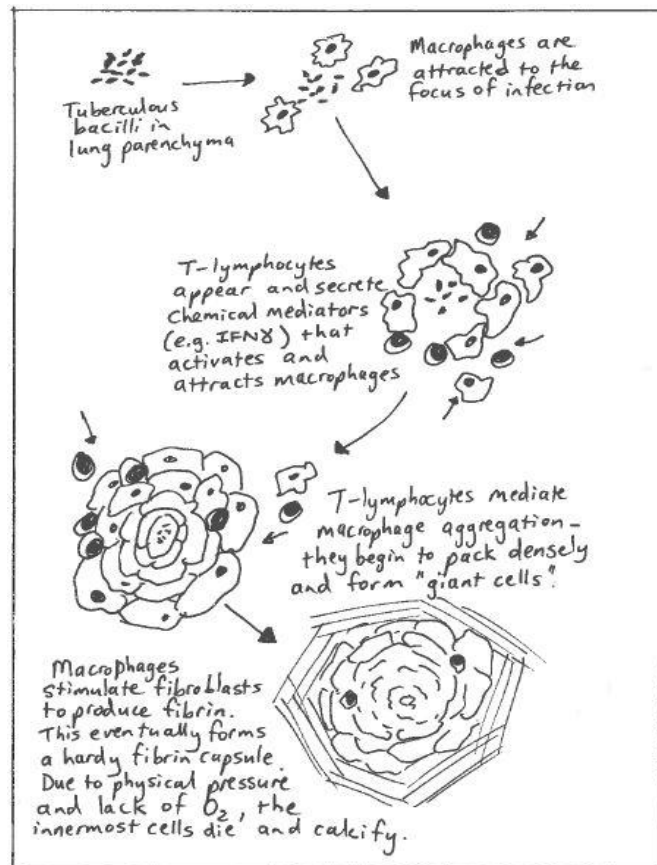


Figure 11 - Granuloma formation

2.6 Immunology of TB

Demonstration of immune reactions in the diagnosis of TB.

The type of immune response which is predominantly elicited during the course of a particular disease depends on the initial interaction of the causative pathogen with the animal's immune system. TB infection and infection caused by other intracellular bacteria such as *Listeriae*, *Brucellae* and *Salmonellae* that survive in macrophages typically induce a cellular response and this limits the initial spread of disease in the animal. In the case of TB, the formation of microscopic and small visible lesions represents the stage of equilibrium between replicating mycobacteria and local cellular host defense. As the host fails to contain the TB organisms and the formation of multiple lesions increasing in size continues, the humoral immune response and production of antibodies is induced. Such antibodies however, do not confer immunity to the disease. For a certain period of time both cellular and humoral immune responses are detectable before the cellular immunity decreases in some animals and eventually disappears (anergic phase, see also 'Anergic reactors').

Among a group of cytokines involved in anti-TB host response, gamma interferon ($\text{INF-}\gamma$) is the principal mediator of cellular immunity. It is released by T-cells of an infected animal upon presentation of mycobacterial antigens such as tuberculin. Both the tuberculin test and the interferon gamma release assay (IGRA) are based on the detection of gamma interferon and its effects.

In contrast, antibodies to *M. bovis* can only be detected by serological techniques. The application of such tests is particularly relevant:

- In TB outbreaks which are not eradicated within a period of several months. It is likely that anergic reactors (infected but tuberculin test negative cattle with advanced disease) have remained undiagnosed but may serve as large scale spreaders of the disease.

If single or very few animals from herds with unknown or positive infection status have to be certified free of tuberculosis, there is a risk of underlying anergic reactions (which fortunately are not common).

3 The Intradermal Test for Bovine Tuberculosis

3.1 Introduction

The tuberculin test is possibly the biological test used most often by veterinary and para-veterinary personnel throughout the world for diagnostic purposes. The test enables one to determine which animals in a herd are infected with bovine tuberculosis. The countries that have already combated and eradicated the disease successfully and efficiently made use of the tuberculin test to identify infected cases.

The test is prescribed by the OIE for international trade and used in tuberculosis control programmes worldwide. There are however, different approaches in the interpretation of test results. Within a country it is important that all officials and private veterinarians use the same standards of testing and interpretation.

It should also be stressed that the test is a herd test and the first and most important diagnosis that must be made is whether the herd is infected or not. Only after this has been established can the individual animal's reaction be assessed.

In South Africa TB is a controlled disease and all testing is done under the control of the Director of Animal Health of the Department of Agriculture, Forestry and Fisheries, and according to the Animal Diseases Act, 1984 (Act No 35 of 1984) and the Animal Diseases Regulations (R.2026 of 1986). Specific regulations in terms of the Bovine Tuberculosis Scheme were also proclaimed in GN R1953 of 30 September 1988.

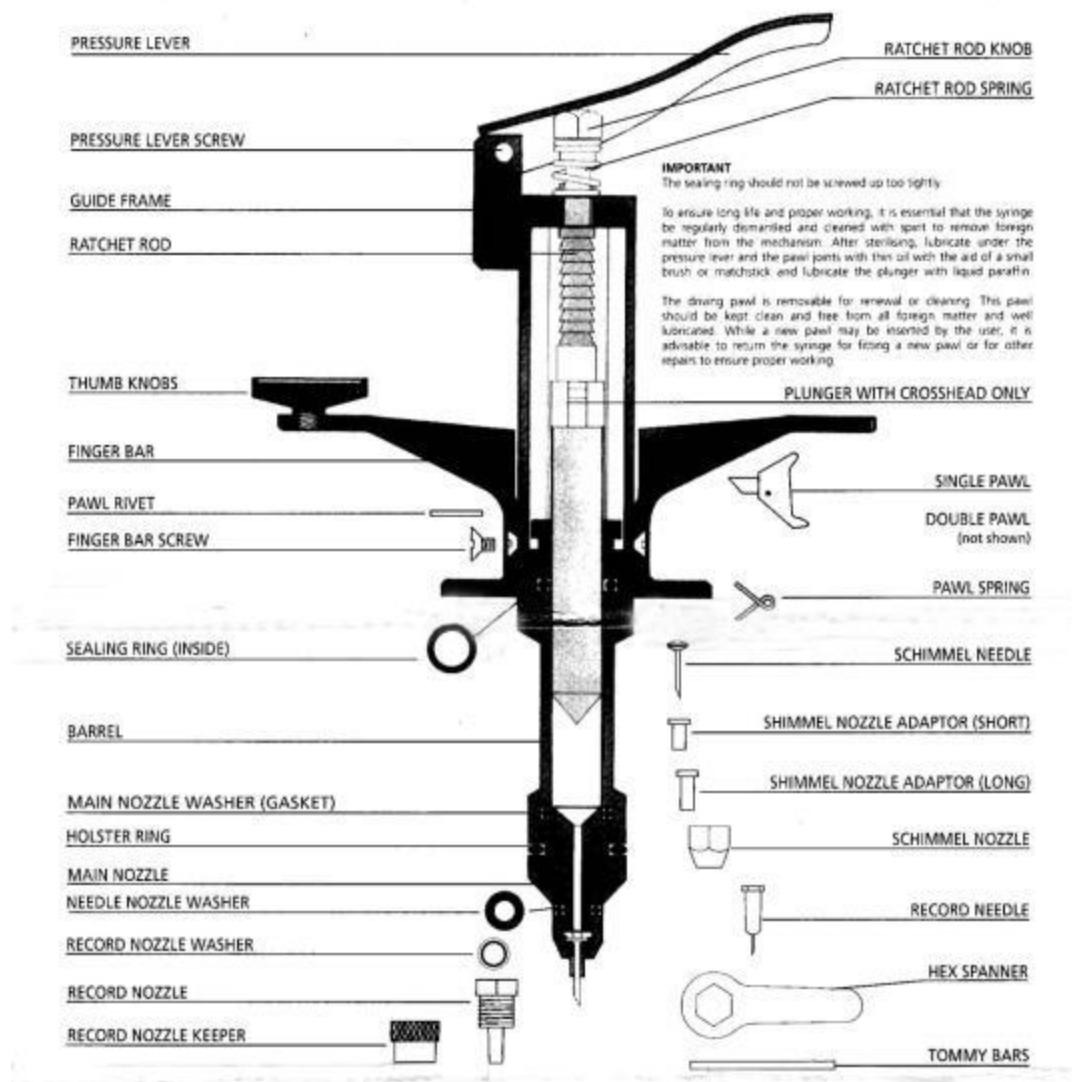


Figure 12 - Parts of McClintock syringe

3.2 Testing Equipment

3.2.1 McClintock Syringe

When testing large numbers of cattle, it is practical and accurate to use the specially designed McClintock syringe to inject 0.1ml of tuberculin intra-dermally. With this device, the tuberculin is definitely injected intra-dermally, whereas with a disposable syringe it may be injected subcutaneously (do not use plastic syringes in bovines). The McClintock syringe is specifically built for TB testing and is relatively robust and automatically injects 0.1ml of tuberculin.



Figure 13 - McClintock syringe and accessories

Before use, the McClintock syringe must be checked and the plunger oiled with small amounts of the lubricant supplied with the syringe or liquid paraffin. The plunger should move smoothly and easily. The syringe should then be rinsed out by filling it with distilled water and injecting this water out. By doing this, the working of the syringe is checked and any leaks or loose parts can be identified and remedied before starting the tuberculin test. Syringes need to be calibrated at regular intervals as recommended by the manufacturer.

Two syringes are needed for the comparative test and the appropriate coloured disks should be placed on the handles to avoid confusion during the test. A third syringe should be available in case of breakage. The syringe with the red disk is used for avian tuberculin and one with the blue disk for bovine tuberculin.



Figure 14 - McClintock syringes showing coloured discs

Once a comparative test has started it is essential to ensure that the syringes are not swapped i.e. the syringe used for avian tuberculin must only be used for avian tuberculin. These syringes are precision instruments and are expensive to replace. They therefore need to be handled with care and maintained on a regular basis. Spares for the McClintock syringes can be ordered from the suppliers. The most common breakage is to the handles. (See fig 12 for parts of the syringe)

Disinfection of syringes

Before using a syringe, test the functioning as described.

Where large numbers of cattle are tested - ideally the syringes should be cleaned and disinfected after every 100 animals (not always practical), or if it has fallen in dung or mud. In the latter case the outside of the syringe should first be rinsed in clean water. The syringe is then filled and rinsed out using sterile water, then filled with 70% ethyl alcohol and left for at least 5 minutes. The alcohol is then ejected and the syringe is again rinsed using sterile water. The rinsing of the syringe should be repeated a number of times to ensure that all traces of the alcohol are removed. At the same time the condition of the needle should be checked and replaced if necessary. If more than one herd is being tested on a day, ideally syringes must be disinfected as above between herds (not always practical).

At the end of the day back at the office, the McClintock syringe must be rinsed externally under running water and then filled out with sterile water to remove all traces of tuberculin, dismantled and sterilized by boiling in water for at least 20 minutes. The rubber O rings and plastic handles must be removed from the syringe before boiling, and placed in 70% ethyl alcohol. After the syringes have cooled, they are reassembled and the plungers oiled with liquid paraffin or the special oil supplied. They are then stored in their cases with the plunger in the middle. A new needle can also be inserted at this stage if necessary.

3.2.2 Needles

Only the special needles (Record or Schimmel) designed for the McClintock may be used. The correct needle is 3.9 mm long (5/32") (Record). This length helps to ensure that the injection takes place in the correct level of the skin and avoids subcutaneous injection. Needles should be examined at regular intervals during a test and replaced if bent or damaged in any way. It is of vital importance to check the needle and syringe if it has been dropped or hit against the crush pen by the sudden movement of an animal. It is important to develop a feel for a sharp needle and as soon as it is felt that the needle is blunt, it should be changed. Never use a needle that has developed a burr on the point or that has been bent or damaged in any way. Do not try to straighten a bent needle, rather replace it.



Figure 15 - Showing bent needle

The number of needles required will depend upon the type used. A short firm needle such as the above-mentioned should last long so that it shouldn't be necessary to have more than ten needles during tests and a supply of five dozen needles at the central office should be enough. The use of types of needles other than those manufactured for the McClintock syringe is unacceptable.

3.2.3 Calipers

To measure the skin thickness, a pair of calipers is used. There are a number of different types available.



Figure 16 - Hauptner calipers being used to measure skin thickness

The Hauptner pistol grip (broad lipped type) with a dial indicator which can measure one tenth of a millimeter is the standard. This instrument has a spring loaded jaw which ensures that a standard pressure is placed on the skin swelling. It does have the disadvantage that this pressure can flatten an oedematous swelling if the reading is not taken quickly enough. They can also be clumsy to use on wilder animals that do not stand still. The pointer on the dial of these calipers moves completely around the dial for every 10mm of skin thickness measured. The slide is also marked at 10mm intervals and this needs to be taken into account when the skin thickness is more than 10mm. Care must be taken when reading large skin increases as it is easy to make a mistake of 10mm. e.g. a skin reaction with a thickness of 25mm will show 5mm on the dial but the markings on the slide would show a position between 20mm and 30mm. the final reading would then be the 20mm shown on the slide and the 5mm shown on the dial = 25mm.

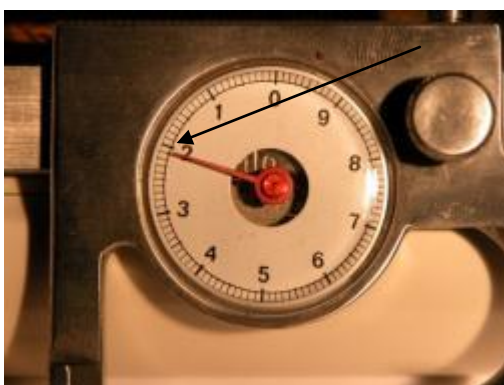


Figure 17 - Dial showing a reading of 12.1mm

Other calipers that have to be opened and closed by hand can be used by testers who have more experience. These are faster and easier to use but it is only with time and experience that testers learn how to apply the same pressure on the swelling every time. With these calipers it is difficult to read in fractions of a millimeter. In order to maintain a standard it is essential that the same person reads both the normal skin and the reaction. Only one instrument is required for tests but it is advisable to have an additional pair of calipers handy in case one is damaged.

3.2.4 Hair Clippers

Before the injection of tuberculin, an area on the side of the neck must be clipped free of hair. This is to identify the injection site as well as to ensure the injection can be done intra-dermally and cleanly.

It is not acceptable to identify the injection site with paint.

Before shaving an area it must be inspected and palpated to ensure that there are not any existing lumps, adhesions or other skin damage that could interfere with the interpretation of the test. The area clipped should be large enough to be visible and allow easy estimation of the centre point for the injection. A square area the width of the clippers is suitable:

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Figure 20 - Re-chargeable clippers and razor

An ordinary double sided razor can be used by testers with experience. This method is however not recommended for general use as it is dangerous to the operator and also can damage the skin at the injection site which complicates the interpretation of the test reaction.

If possible – do not clip area where there is no pigmentation as sunburn may affect results.



Figure 21 - Damage caused by razor

3.3 Other Equipment

Clipboard:

It is essential to have a clipboard to hold writing paper and the TB 10 forms. The clipboard should have a pen or pencil attached to it.

Cotton Wool:

Cotton wool is handy to clean the injection site if it is soiled. It should be used dry if possible. If the injection site is so dirty that it needs to be washed, then the cotton wool is used to dry off the site. The injection site must be clean and dry before injecting tuberculin.

Disinfectant:

Usually no disinfectant is used on the injection site. If the site is very soiled, then it can be disinfected using ethyl alcohol (or methylated spirits). If the site is disinfected then it must be given enough time to dry completely before the tuberculin is injected. Bottles containing 70% ethyl alcohol and sterile water must also be available as standard materials to disinfect syringes during the test as described under 3.2.1.1 above.

Fold-up Table:

A sturdy fold up table is essential to enable equipment to be kept off the ground and can also be used for writing of notes and completion of TB 10 forms.

Tool Box:

A sturdy steel or hard plastic box to hold all the equipment needed for tuberculin testing should be used. This not only protects the equipment, but also ensures that all equipment is kept together. A record of the contents of the box should be taped to the inside of the lid. This assists with the checking and packing of equipment after a test is completed.

Umbrella:

Most tuberculin tests are performed outdoors, often without any shade. An umbrella provides shade for the equipment and cool box, thus ensuring the correct temperature is maintained. It also provides shade for officials whilst waiting for animals to arrive.

Cooler Box:

A cooler box is an essential part of testing equipment and the tuberculin must be kept between 4 - 8°C in the dark during the test. The cooler box should be large enough for the amount of tuberculin needed for the testing and sufficient ice packs should be available to ensure the maintenance of a temperature of 4 – 8°C at all times. NOTE: Tuberculin bottles should be surrounded by, but not in direct contact with ice packs to avoid freezing!

Checklist:

A checklist is provided as an annexure to this manual. This can be reproduced and used in the field.

First Aid Kit:

Performing a TB test can be dangerous and officials should always carry a basic first aid kit so that minor injuries can be correctly treated.

Pliers & Thin Screwdriver:

Always good to have for assisting in fixing McClintock syringes and thin screwdriver also useful for assisting in filling McClintock from the 18 dose vials.

3.4 Animal Handling Equipment

The normal animal handling equipment should be available during tuberculin testing, i.e. nose tongs, ropes, suitable crush pen and neck clamps. It is often difficult to perform a tuberculin test on animals restrained in a neck clamp as it interferes with access to the side of the neck. This should only be used for animals that cannot be tested in the crush. If cattle are correctly and firmly packed into the crush pen then the shaving and injection should be possible with few problems.

Facilities on farms vary greatly and care must be taken to ensure both the safety of the persons testing as well as to ensure that the test can be performed properly.



Figure 22 - Farm with excellent facilities



Figure 23 - Farm with reasonable facilities



Figure 24 - Animal in neck clamp

3.5 Tuberculin

Bovine tuberculin PPD (purified protein derivative) is prepared from protein contained in the culture filtrate of the *M. bovis* tuberculin production strain AN5. When injected into the skin of a bovine infected with *M. bovis*, this tuberculin causes a delayed hypersensitivity reaction. This manifests as an inflammatory reaction called a tubercle follicle at the site of injection and is the basic lesion responsible for the typical signs seen in test-positive animals.

According to the OIE Terrestrial Manual 2009, a dose of tuberculin injected must be no lower than 2000 International Units (IU) of bovine and avian tuberculin. The OIE further advises that in cattle with diminished allergic sensitivity, a higher dose of bovine tuberculin is needed and in national eradication campaigns, doses of up to 5000 IU are recommended.

All tuberculin is issued with a G-number (registration), batch number and an expiry date. These must be checked and recorded before the performing a test. **NB:** Tuberculin must never be used after its expiry date!



Figure 27 - German Avian tuberculin



Figure 28 - German 18 dose bovine tuberculin vials



Figure 29 - 20 dose bovine tuberculin

3.6 Testing Procedure

3.6.1 Selecting the Type of Test

Before a test is organized the history of the herd involved must be determined and recorded.

The **comparative intradermal test** is generally used for herds:

- where the TB status or the history of the herd is unknown or vague
- where non-specific reactions occur
- where problems are experienced with the interpretation of tests

The simultaneous use of both the avian and bovine tuberculin is therefore mainly a diagnostic aid to obtain a more definite diagnosis in respect of the presence or absence of bovine TB in a herd.

The **single intradermal test** on the other hand is mainly used where:

- the negative TB status of the herd is known;
- animals are tested for the first time (depending on the history);

Positive herds can be tested with the single intradermal test, depending on the previous reactor rate and the use of additional tests such as gamma interferon test and serology.

3.6.2 Site of Injection

It is known that the skin of an animal does not show the same degree of sensitivity to tuberculin everywhere on the body. There is an increase in sensitivity from the back towards the front and from the bottom to the top of the body. A sensitivity of 1 in the skin of the hindquarters increases to a 3 in the neck.

The same dosage of bovine and avian tuberculin is injected during a test. It is therefore important to inject both avian and bovine tuberculin in an area with the same sensitivity. This will prevent the sensitivity of the skin from having an effect on the interpretation of the test.

CERVICAL TEST: The tuberculin is injected on the side of the neck, midway between the head and the shoulder and halfway between the top and bottom of the neck. **In South Africa this is the only approved site for intradermal TB testing in cattle.**

a.) Single Intradermal Test

A place on the side of the neck halfway; between the juncture of the head and neck and the fold in front of the shoulder and halfway between the top and bottom of the neck is chosen. The place is palpated to determine whether the skin texture is normal without lumps in or under the skin and to ensure that there are no adhesions between the skin and the subcutaneous tissue. It is necessary that all the animals of one herd be tested on the same side, be it on the left or the right hand side.

b.) Comparative Test

When a comparative test is being performed then two sites on the same side of the neck need to be prepared. The bovine tuberculin is injected closer to the shoulder, whereas the avian tuberculin is injected closer to the head. The two sites should be at least 15cm apart and both sites must be palpated and found free of lumps, scars or adhesions. It is sometimes recommended that the avian tuberculin is injected on the opposite side of the neck. This method is difficult to perform under field conditions. For practical purposes preference is given to the method where both tests are performed on the same side of the neck.

c.) Disinfection of Syringes

Before using a syringe test the functioning as described.

d.) Filling of Syringes

With old type tuberculin bottles, as well as new type bovine tuberculin bottles:

Filling syringe from German vials:

- When all the tuberculin is in the syringe, then one dose is injected back into the bottle. This should appear as sharp solid jet of fluid. If there is any air in the syringe then the air can be seen as it is injected into the bottle. If air is present then extra doses must be injected into the bottle until no air is present in the syringe at all.

e.) Injecting Tuberculin



Figure 30 - Correct angle to inject

Potentially complicating factors:

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- The correct site of injection is into the lower layers of the epidermis; in this case there is a measure of resistance when injecting and the animal often shows a pain reaction. This site will also cause a pea sized nodule in the skin. It is essential that after every injection, the site is gently palpated to ensure that this nodule is present. If no nodule can be felt the injection has not been made correctly and must be repeated.
- Do not inject into unpigmented skin if possible to avoid sunburn reaction

After the tuberculin is injected the test is read (interpreted) after a period of 72 hours (3 days) without exception, otherwise the test will have to be repeated after 3 months. There are cases where the reaction peaks earlier or later than 72 hours but these are the exception and not the rule. If a large oedematous swelling is present it may have lost a lot of fluid before the 72 hour reading and will be smaller. However this reaction will still be interpreted as a positive reaction after the 72 hours.

A diagnosis is not made only on the skin measurement. Every reaction must be examined and fully described. The difference between reading the results and the interpretation must be understood. Reading the results means looking, feeling and measuring the reactions and then fully describing them. Interpretation is done after the whole herd has been examined and all the reactions are taken into account. Using this information the decision is made as to the status of the herd.

NB: Animals must not be allowed to run through the crush and only a visual inspection done. Every animal must be held in the crush and each injection site must be examined and palpated. If there is any sign of a reaction in any animal then the skin measurement must be taken.

It is essential when reading a test that every single animal that was injected is again present on the day of the reading. This emphasizes the importance of identification of animals and proper recording of these numbers on the day of injection.

- Observation
- Feeling (Palpation)
- Hearing
- Measurement

- The appearance of the swelling may be round or flat.
- The swelling may be clearly demarcated from the surrounding normal skin (circumscribed) or gradually runs into the surrounding tissues with no clear boundary (diffuse)
- Colour changes such as blue and red may be seen in light coloured skin

- ### 3.7.2 Palpation

- Consistency, hard or soft swelling
- Oedema
- Heat
- Pain when site is palpated
- Adhesions between the skin and the subcutaneous tissues
-

3.7.4 Local Reactions

This is indicated on the TB 10 form by the abbreviation O. Oedema is the abnormal accumulation of fluid in a tissue and can be felt as a soft or hard swelling. If it is pressed then a pit remains when the pressure is released. This is called pitting on pressure. Oedema can be felt when the lesion is palpated, it may also be circumscribed or diffuse. Oedema is a very positive sign and almost always indicates infection. In an infected herd, oedema should always be considered to indicate a positive reaction, even if the skin thickening indicates a negative reaction.



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c.) Necrosis

The reaction must be described on the TB 10 form. Necrosis (dying off of cells) can be seen as a round dark area in the centre of the reaction. It is normally surrounded by an area of intense inflammation. The necrosis is caused by the swelling of the tissue and the interruption of blood supply to the central areas of the reaction. Necrosis is regarded as a strongly positive sign and is often seen in animals that have recently become infected and which show a severe tuberculin test reaction.

d.) Exudation

The reaction must be described on the TB 10 form. Exudation is the leaking of fluid from the swelling. Furthermore, exudation of fluid may reduce the size of the skin thickening and is always regarded as a strongly positive sign. Note that an exudate may dry on the skin surface and then it appears as a dry scab or clot on the skin surface.

e.) Lymph ducts/nodes

Reactions involving lymph vessels and nodes must be described on the TB 10 form. The lymph nodes of the area next to the reaction may be enlarged and painful in the case of infected animals. The lymph ducts draining the area are swollen and may easily be seen. The hair over the swollen lymph ducts stands erect, making them easily visible. The **pre-scapular lymph node** should be firmly palpated between the fingers and the thumb. The size of the lymph node on the side of the tuberculin injection should be compared with the lymph node on the opposite side of the body. Many animals will show a pain reaction when a normal pre-scapular lymph node is palpated. The involvement of the lymphatic system is a very positive sign.

f.) Pain (T) (Dolor)

This is indicated on the TB 10 form by the abbreviation T (Tender). During the examination of an animal that shows a reaction, the swelling is also handled and palpated. If the animal stands still and shows no signs of uneasiness it can be accepted that the swelling is not tender. However, if the animal moves about and pulls away this may indicate that the swelling is painful. The measurement of pain is very subjective as some animals will show signs of pain when normal skin on the neck is palpated. This can often be tested by first handling an area of normal skin and then the reaction site. The reaction of the animal can be compared and an indication obtained whether the swelling is painful or not. Because of the subjective nature of this sign, it cannot be relied upon too much when making a diagnosis.

g.) Heat (H) (Calor)

This is indicated on the TB 10 form by the abbreviation H. There is no practical mechanical method of determining whether the swelling is warmer than the normal skin. The only subjective way is to compare the reaction site with an area of normal skin away from the injection site. This is still a very subjective method and as most TB tests are carried out in the open and sunshine, the animal's skin may be normally very warm. If there is without doubt an increase in heat at the reaction site, it is a positive sign, but in most cases heat cannot be used to assist in making a diagnosis.

3.7.5 Systemic Reactions

In an animal with advanced BTB, signs such as coughing or signs of pneumonia will sometimes occur one to two days after the injection of the tuberculin. The following signs may also be seen:

- Increase in temperature (fever)
- Shivering
- Ruffled hair
- Listlessness
- Decreased production in lactating cows

3.7.6 Measurement

The caliper is placed in position over the thickest part of the swelling and the lips of the caliper moved into position until they touch the skin in such a way that the same pressure is exerted as when measuring the normal skinfold without disturbing and flattening the swelling. The skin reading is only measured in millimeters and tenths of millimeters for the sake of uniformity. This reading is noted, as well as the abbreviations describing the reaction. An individual diagnosis is made and noted after all the tested animals have been examined and a decision made on whether the herd is infected, suspect or negative.

- The normal skinfold is measured above the place of reaction or on the opposite side of the neck where tuberculin was not administered and noted on the TB 10 form.
- The thickened skinfold is measured at the thickest place(s) of injection reaction and noted on the TB 10 form.
- The type of reaction is observed and palpated at the site of injection of bovine tuberculin and described by making use of the recognized abbreviations.

No measurement is made and no description of the reaction site is filled in on the TB10 form where there are no palpable reactions at the injection site after 72 hours. The fact that no skin thickening readings need to be given in respect of cattle that show no reaction to the injection of tuberculin does not indemnify the tester against the fact that each injection site regardless of the testing program must be examined and palpated at the time of reading the test. **It is however of vital importance that all signs are taken into account when interpreting a test result.**

The following criteria are used to describe the swelling along with the increase in skin thickness, as well as those already mentioned above under section 3.8.4.

Describe in the test report as “Hard”. The swelling feels like a firm round nodule. Other signs are normally absent and these hard circumscribed nodules are often caused by non-specific reactions.

This is described on the TB 10 form as C. There is a very clear demarcation between the reaction zone and the normal skin. This is seen as a distinct line separating the two. This type of swelling often occurs with a hard cold non painful reaction and is often caused by non-specific reactions. It can also occur in infected animals with necrosis and oedema.



Figure 32 - Circumscribed lesion with sharply defined edges



Figure 33 - Small hard, circumscribed reaction

c.) Diffuse (D)

This is described on the TB 10 form as D. If the reaction zone gradually runs into the surrounding tissue, it is diffuse. It is normally associated with oedema and indicates towards a positive reaction.



Figure 34 -Diffuse type swelling with no distinct edges

d.) Flat (F)

This is described by the abbreviation F. When a reaction is flat and one looks at it without handling or measuring it, there is a danger that it could be missed. If a flat reaction is hard and cold, infection is not expected but it can also be associated with oedema and other signs as shown below, when it will be associated with infection and this is one of the reasons why it is so important to palpate every single animal when reading a test.



Figure 35 - Flat, diffuse, oedematous swelling

e.) Adhesions (Ad)

This is described by the abbreviation Ad. In typical positive reactions there are often adhesions formed between the skin and the subcutaneous tissues. The adhesion can be felt when palpating a reaction by trying to lift the skin away from the body. Because adhesions are a very positive sign it is essential to palpate the site before injection to ensure that there are no adhesions present before the test.



Figure 36 - Palpation of swelling with no adhesion

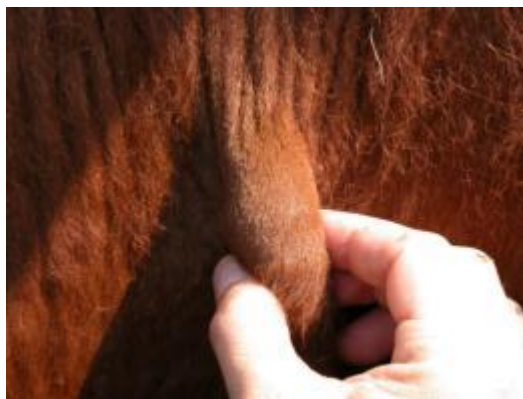


Figure 37 - Palpation of swelling with adhesion

3.8 Interpretation of Test Results

3.8.1 Herd Approach

The test is conducted on the whole herd. The only exception is when a small number of animals have to be tested for diagnostic or export purposes, in which case these should preferably be carried out by a private

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practitioner at the owner's expense. The interpretation takes place on a herd basis and all animals showing reactions must be held back until the interpretation is complete. All the reactions in the herd are now taken into consideration. It will now be possible to determine the herd status. Only after the herd status is determined can the interpretation of individual animal reactions be performed. When reading a herd test it is of value to walk through the cattle before they are packed into the crush pen. By doing this large severe reactions can be seen and should be placed in the crush pen first as this helps to determine the status of the herd. The general body condition of the cattle including external parasite load, as well as the overall quality of herd management can provide additional useful information towards a final diagnosis.

NB: Remember that all signs, history and the possibility of non-specific reactions must be taken into account. Do not rely only on the increase in skin thickness.

3.8.2 Herd History

If the history of the herd is known this will simplify the interpretation. The following information is of great value:

- Results of previous tuberculin tests
- Post mortem/abattoir results on cattle, pigs and poultry
- Closed herd or not
- Recent introductions of cattle
- TB bacteria found in milk (culture)
- Avian TB found in poultry or feeding chicken litter
- Human TB in farm workers
- Johne's disease diagnosed on the farm
- Skin lesions in the herd
- Clinical suspect or positive cases
- History of TB in contact farms
- Systemic reactions after doing TB test
- Drop in milk production since doing TB test herd milk normally drops after TB test – Interfere with cows normal routine

On the basis of the history, herds can be provisionally divided into three groups:

- Negative for TB
- Possibly negative but uncertain TB status
- Infected with bovine TB

3.8.3 TB Negative herds

There will be herds where the above-mentioned data are not available. Such herds are regarded as being TB negative until the contrary is proven. Maintenance herds where animals are tested regularly and where it is ensured that additions are tested negative before they may mix with the herd. **Previously** infected herds where the disease has been eradicated by means of regular tests, the elimination of reactors and disinfection.

3.8.4 Tuberculin test interpretation in a suspected negative herd

It should be stressed again that a final diagnosis on the status of a herd should not only be made on the increase in skin thickness. This is only one of many factors to be taken into account. The possibility of non-specific reactions must be taken into account as well. The age of the animals tested also needs to be considered. Animals under 12 – 18 months tend to show more non-specific sensitivity in respect to environmental *Mycobacteria*. Animals older than 5 years have a higher chance of being infected for a long time and may be completely insensitive (anergic) to the tuberculin test. Skin lesions also cause most non-specific sensitivity in animals between 2 – 5 years old. The same skin thickening in millimeters in animals with a thin normal skin is of greater significance than in animals with a thicker skin. The density of the skin can also have an influence on the reaction, particularly in respect of fluid accumulation (oedema).

a.) Single Intradermal test with bovine tuberculin only (supposed negative herds)

Negative Herds

In a herd with no history of TB infection, especially in closed herds, it can be expected that most animals will be negative. Where animals show skin thickenings after 72 hours a reason will be looked for i.e. non-specific reactions, or the possibility of human TB infected animals etc. In these herds an increase in skin thickness of up to **6mm** is regarded as negative. Once again the type of reaction must also be considered. A herd with a negative history showing a number of hard circumscribed reactions, even with increases of up to 6 mm or more can be regarded as negative. If there is any doubt as to the status of the herd it must be retested after three months using the comparative test or gamma interferon test.

Suspect Herds

When a herd is tested for the first time and a number of reactions are found with an increase of more than 6mm but with signs of a positive reaction, i.e. oedema, necrosis, exudation should be regarded as a suspect herd and retested in 3 months using the comparative intradermal test. Alternatively, blood samples in heparin can be collected from the suspect animals and submitted to the laboratory for gamma interferon testing.

Positive herds

When large typical reactions are found in a number of animals in a herd tested for the first time and a few animals show increases of more than 20mm, the herd can be regarded as positive. The positive reactors should be slaughtered for post mortem examination and affected tissue samples sent to the laboratory to confirm the diagnosis. Infection of animals with *M. tuberculosis* can in some cases also give this picture and the possibility of sensitization by infected workers should be eliminated first. Such animals, if tested after 3 months, usually show a decrease in skin thickness but in some cases a positive test result may persist.

b.) Comparative intradermal (negative herds)

In interpreting the comparative test two extra criteria are used in addition to all the normal criteria as described in Section 3.8

- The increase in skin thickness at the bovine tuberculin site
- The difference between the bovine and avian increases (the avian increase is subtracted from the bovine increase)

The table below shows the interpretation of the comparative test in negative or suspected negative herds.

A positive reaction will be characterized by the following measurements:

- A bovine increase of more than 4mm
- A positive difference in increase of skin thickness between bovine and avian injection sites of more than 4mm.

Skin thickening in millimeters		
Bovine	Bovine minus avian increase	Diagnosis
Less than 4	Less than or equal to 0	Negative
More than 4	1 to 2 mm	Negative
More than 4	3 to 4 mm	Suspect
More than 4	More than 5	Positive

Examples:

Scenario 1

Normal skin 10mm
Bovine reaction 13mm
Bovine increase 3mm
Avian Reaction 18mm
Avian increase 8mm
Bovine minus Avian ($3 - 8$) = -5
Therefore **Negative**

Scenario 2

Normal skin 10mm
Bovine reaction 18mm
Bovine increase 8mm
Avian Reaction 12mm
Avian increase 2mm
Bovine minus Avian ($8 - 2$) = 6
Therefore **Positive provided** others signs point towards positive reaction.

It is evident from the above that where the avian increase is greater than or equal to the bovine increase then a diagnosis of negative is made.

In cases of (non-specific) avian reactions the avian reaction is much larger than the bovine reaction.



Figure 38 - Animal showing reactions at both bovine and avian injection sites

3.8.5 Causes of non-specific (false positive) reactions

The purpose of the tuberculin test is to trace animals that are infected with *M. bovis* (bovine tuberculosis) and to differentiate such animals, if possible, from other who do in fact have a thickening of the skin after the tuberculin test but who are not infected with *M. bovis*.

Generally non-specific reactions are caused by temporary sensitization with non-pathogenic mycobacteria. In most cases re-testing of animals after 3 months produces a conclusive result. The purpose of the comparative tuberculin test is also to differentiate animals that show some reaction to the tuberculin test but are not infected with *M. bovis* from those which are infected with *M. bovis*. In most cases non-pathogenic mycobacteria will cause a strong reaction to avian tuberculin PPD and can be readily identified using the interpretation key as provided in this manual. Due to the close antigenic relatedness among mycobacteria it is possible, however, that:

- Non-specific stimulation results in a temporary significantly stronger reaction to bovine tuberculin PPD.
- Specific stimulation by *M. bovis* infection results in temporary excessive reaction to avian tuberculin PPD.
- A mixed infection with both *M. bovis* and non-pathogenic mycobacteria results in temporary excessive reactivity to avian tuberculin PPD.

a.) Mammalian reactions

b.) Avian type reactions

Johne's Disease - Infection with *M. avium* subsp. *paratuberculosis* is rare in cattle in South Africa, but the disease is widespread in sheep flocks in several provinces in the country. Infected animals that are tested with bovine tuberculin usually do not show typical reactions but more of a suspect type of reaction. Sensitivity in respect of the tuberculin test is usually the greatest immediately after infection has taken place, thereafter it decreases and animals with noticeable symptoms will probably react poorly or not at all to the bovine tuberculin. *M. paratuberculosis* is antigenically related to *M. avium* and accordingly elicits greater reactions to the avian tuberculin than to the bovine tuberculin.

c.) Non-Mycobacterium reactions

Nocardiosis – The acid fast *Nocardia spp.* can cause problems regarding the differential diagnosis of TB and cultures of *Nocardia* can be confused with mycobacteria. Histopathologically the lesions caused by these organisms can be similar to TB and a degree of cross sensitivity can be experienced between these organisms and mammalian and avian tuberculin which in experimental cases disappears after 72 days. A retest after 3 months will eliminate *Nocardia spp.* as a possible source of sensitivity.

- Actinomycosis
- Actinobacillosis
- Brucellosis
- Corynebacteriosis
- Trichophyton infections

Liver fluke infection, peritonitis, abscesses, cysticercosis, pneumonia, mastitis, nephritis, and lumpy skin disease are sometimes blamed as causes of non-specific sensitization. However these are not proven and the reactions caused will show a decrease on repeated testing. Herds with a liver fluke infestation should be dosed before the next test, to eliminate liver fluke as a potential problem.

In the advanced stage of pregnancy or during the peak of the oestrus cycle cows sometimes show an increased sensitivity to the tuberculin test. Non-infected bulls between the ages of 2 - 4 years show up to 3 times more suspect reactions than non-infected cows of the same age. This increase in sensitivity is attributed to the influence of sex hormones.

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the tuberculin. The cause of these local reactions is often obvious on closer examination of the area.

3.8.6 Infected herds

a.) Herds are regarded as infected if any of the following examinations indicate infection

i) *Meat Inspection*

When lesions typical for infection with bovine TB are found during routine meat inspection at an abattoir, it must be immediately reported telephonically to the State Veterinarian in whose area the cattle originated as well as on the meat inspection form in terms of the Meat Safety Act, 2000 (Act No. 40 of 2000). The State Veterinarian is responsible for the testing of the infected herd and the tracing of all movements. After a positive meat examination the testing of the herd is compulsory.

ii) *Infection in milk*

Some municipalities and distributors conduct random sample testing of milk. If acid fast organisms are detected in a milk sample then testing of the herd of origin by the State Veterinary Services is compulsory.

iii) *Post Mortem examination*

Infection can also be detected when post mortem examinations are conducted on the farm, either by the State Veterinarian or a private veterinarian. If infection is suspected by a private veterinarian then it must legally be reported to the local State Veterinarian. Testing of such a herd is compulsory.

iv) *Clinical cases*

Clinical cases may be detected by Veterinarians or Animal Health Technicians during routine inspections. Old animals with a cough and emaciation should be suspected of being infected.

v) *Tuberculin test*

The intra-dermal tuberculin test is the most common way in which TB is detected in animals. This test may be performed by state officials or by private veterinarians. If a positive reaction is found by a private veterinarian, then he/she is legally obliged to report this immediately to the local state veterinarian. The state will then take control of that herd and will remain responsible for all testing and other control measures until the herd is declared negative again.

vi) *Introduction of animals from an infected farm*

When infection is confirmed on a farm, all movements of animals off that farm must be investigated. Any farm that has received animals from the infected farm must be regarded as infected and the state veterinarian of the area of destination must be informed. The testing of such farms is compulsory and such herds are regarded as positive until proven negative.

b.) Interpretation of single intradermal test (positive herds)

A number of animals in an infected herd, will only have become infected fairly recently. This means that they will show large reactions to the tuberculin test. Such animals will show typical reactions with skin thickening of more than 20mm with some or all of the following positive signs:

- Oedema
- Pain
- Redness
- Necrosis
- Adhesions
- Enlargement of pre-scapular lymph node

In an infected herd the average of all skin increases will normally be 10 - 12 mm.

****Once the herd has been declared positive, then all animals with a skin thickening of 4mm or more on the bovine tuberculin are regarded as positive.**

The following exceptions to the above should be taken into consideration:

- Old animals with a skin increase of less than 4mm but with other positive signs, i.e. oedema, pain, heat, etc.
- Old animals with skin increase of less than 4mm with hard and circumscribed lesions but with clinically suspect signs i.e. coughing, emaciation, swollen lymph glands, etc.
- Animals younger than 5 years with a skin thickening of 4mm and more where the reaction is circumscribed and without heat. With such animals a diagnosis of suspect can be made and the animals retested after three months. These animals should, ideally, be kept separate from the rest of the herd until their status is confirmed.

c.) Interpretation of comparative tuberculin test (positive herds)

Skin thickening at injection site(s) in mm		Diagnosis
Bovine injection site	Bovine reaction minus avian reaction	
Any	Less than or equal to 0	Negative
4mm or more	Plus 1 or plus 2	Suspicious
4mm or more	Plus 3 or greater	Positive

The exceptions mentioned above must also be considered in the interpretation. Old animals with a suspicious diagnosis can be regarded as positive if the bovine reaction shows any other positive signs, i.e. oedema, heat, etc. Animals up to 5 years old with a positive interpretation according to the table above may be considered suspicious if the bovine reactions are hard, circumscribed with no pain or heat, and there are indications that other causes of sensitivity are present on the farm. This would include skin lesions, liver fluke etc.

The success and speed with which TB will be eradicated in an infected herd is closely related to:

- The ability of those doing the tests to identify all infected animals and to remove these from the herd as soon as possible; and
- Adherence to strict testing schedules of the entire herd.

The stricter the interpretation in the first positive test, the quicker the disease will be brought under control. At the beginning of a control operation, caution must be exercised in making a diagnosis of suspect animals, it is often better to remove such animals from the herd. If they are kept for retesting in three months, they should be kept in a separate herd until their status is confirmed.

Note: Successful eradication of an outbreak requires the synergistic application of available diagnostic and epidemiological tools to identify infected cattle but also to identify and analyze other factors which can potentially complicate the outbreak.

In a positive herd, record keeping is essential and all animals have to be identified with ear tags or brands. This allows the State Veterinarian to follow the individual animals through the testing procedure and improves the accuracy of the interpretation.

In controlling the disease in any farm, buy in and cooperation from the farmer is essential and the better the cooperation, the faster the disease can be eradicated. Ideally cooperation should be achieved by positive

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3.8.7 Causes of false negative tuberculin reactions

These are cases where the animal has bovine TB infection but does not react to the tuberculin test.

Infection with *M. bovis* causes the largest and most typical reactions in the early stages after infection. This sensitivity lessens later on during the advanced stages of the disease. As the immune system of infected cattle gradually switches from a cell-mediated type (which is primarily detected by skin test and gamma interferon test) to a humoral antibody-based reaction, the skin test becomes less sensitive in detecting infected animals. If the eradication campaign fails to remove such animals in time or in the absence of regular routine testing, they will eventually become totally non-responsive to the skin test (no reaction at all). This is known as anergy. These anergic reactors often show generalized spread of TB throughout the body and are therefore a great danger and source of infection to the rest of the herd.

Energy is more often found in the older animals and special attention must be given to this group. The following tests can be applied to try and identify these animals:

- b.) Inactive TB

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After an animal has become infected it takes from 8 – 65 days (average 21 days) before an allergic condition develops and the animals react to the tuberculin test. These animals will however be detected at the following TB test in three months' time.

The application of illegal practices such as the injection of cortisone and other drugs causing vaso-constriction could affect the interpretation of the tuberculin test.

Shortly before and after calving infected cows may show a false negative skin test result. Such animals will return to a positive test status four to six weeks later. Such doubtful reactors should be retested after three months.

Considering all that has been said and taking into consideration skin thickening, reaction signs, the history of the herd and other information, the following table may help to give a better idea of how a combination of skin thickening and other information can be used to come to a decision whether the animal concerned is positive, suspect or negative. On the left hand side of the median column of the table are the signs that tend to be positive and on the left hand side are the signs that indicate a negative diagnosis.

For a specific type of herd (negative or positive), the number of millimeters skin thickening which is the recommended differential between positive or negative are placed on the median according to the type of test (single or comparative) to distinguish between negative or suspect on the one hand and negative/suspect or positive on the other. As far as a specific animal is concerned the reaction signs and other data are then taken into consideration and a decision is made whether the evidence, as far as that animal is concerned, should be put on the left hand side of the median that is to say on the positive side or right of the median that is to say on the negative side. This helps to decide on a positive, suspect or negative diagnosis.

Type of herd, skin thickening, median and proposed diagnosis

Type of herd, skin thickening, median and proposed diagnosis			
	Greater	Median **	Smaller
For a negative herd			
Bovine tuberculin alone	Suspect/ Positive	> 6mm <	Negative
Bovine & avian tuberculin	Suspect/ Positive (Provided the reaction to bovine tuberculin is 4mm or more)	> + 5mm < (difference)	Negative
For a positive herd			
Bovine tuberculin alone	Positive	> 4mm <	Negative or Suspect
Bovine & avian tuberculin	Positive (Provided the reaction to bovine tuberculin is 4mm or more)	> + 3mm < (difference)	Negative or Suspect

**Median column - recording the diameter of skin thickening in mm.

Positive signs	Negative signs
1. Skin thickening - large	1. Skin thickening – small or negligible
2. Herd history – positive for TB	2. Herd history – negative or unknown
3. Pain = T	3. Hard
4. Heat = H	4. Circumscribed = C
5. Skin colouring – red, blue, etc	5. Flat = F
6. Oedema = O	6. Skin lesions – see or feel
7. Functional skin changes such as: 7.1 Necrosis = Nec 7.2 Exudation = Ex 7.3 Lymph nodes & lymph ducts swollen = Lnn or Ld	History of: 7.1 Avian tuberculosis on farm – <i>M. avium</i> 7.2 Feeding of chicken litter 7.3 Human tuberculosis – <i>M. tuberculosis</i> 7.4 Johne's disease – <i>M. paratuberculosis</i>
8. Systemic reactions such as: 8.1 Fever 8.2 Shivering 8.3 Dull hair coat 8.4 Listlessness 8.5 Decreased production in dairy cows 8.6 Coughing spells	8. Young animal
9. Adhesion of skin to subcutaneous tissue = Ad	
10. Old animal	
11. Swelling diffuse = D	

4 The Gamma Interferon Assay (IFN- γ)

4.1 Principle

The IFN- γ test is, like the tuberculin test, a method to detect an animal's cellular immune response to *M. bovis*. It is therefore in essence an *in vitro* analogue of the tuberculin test.

4.2 Procedure

Samples (10ml) of whole blood in heparin (green top tube) are taken aseptically from individually identified bovines. The blood samples are transported to a laboratory equipped to perform the preparatory stimulation (first phase) of the test. During transport the blood samples are kept between 15°C and 25°C (NO chilling). As the stimulation relies on viable T-cells this step must be done on the day of sample collection. In the laboratory the blood samples are aliquoted and stimulated with bovine and avian tuberculin as well as Fortuitum PPD (available from the OVI TB laboratory). Following incubation at 37°C for 24 hours the clear plasma supernatant is harvested in the laboratory and can be assayed for the presence of gamma interferon (second phase). The entire duration of the test is approximately 2 days. Note that the samples need to be processed within a maximum of 4-6 hours after being collected (this is often a limiting factor for testing).

4.3 Use of the IFN- γ test

The IFN γ test is not intended to replace the tuberculin test because of higher costs in most cases but it is useful as an ancillary test to the tuberculin test in all situations where a sensitive and quick diagnosis is required. A final diagnosis of suspect reactions detected during routine tuberculin testing can be obtained as early as 2 weeks after

the skin test.

The major advantages of the IFN- γ test can be summarized as follows:

- Animals are handled only once, therefore only one farm visit necessary
- No waiting period between IFN- γ tests
- Test performance is not related to experience of field staff (as for the tuberculin test)
- No unlawful intervention possible (use of drugs, swapping of ear tags etc.)
- IFN- γ test has the capability of detecting early infections. Parallel use with tuberculin test therefore result in increased overall diagnostic sensitivity

5 Zoonotic Aspects

TB in humans (mainly caused by *Mycobacterium tuberculosis*) is a very important disease and has a worldwide distribution. TB is common in situations of poverty, malnutrition, densely communities and poor hygiene. Bovine tuberculosis (*Mycobacterium bovis*) is a zoonosis and although it is mostly contracted via infected, unpasteurised milk, it can also be transmitted by aerosol during close contact between humans and infected cattle (e.g. handling, slaughter). The susceptibility of humans to TB must be considered higher in immuno-suppressed patients infected with HIV.



Figure 39- Informal settlement

TB has been almost eradicated in humans in Europe through pasteurization, immunization and improvement in socio-economic status. *M. bovis* and *M. tuberculosis* cause similar lesions in humans. They can only be differentiated by means of specialized culture or molecular identification techniques. Close contact with livestock and the consumption of unpasteurized milk and milk products should alert medical authorities to the possibility of livestock as a source of TB to the individual/ community. Adequate control of *M. bovis* in livestock (and wildlife) will prevent the spillover of this organism into the human population. During the medical diagnosis of TB in people, *M. tuberculosis* and *M. bovis* are not always differentiated, leading to a potential misperception of the original source of the disease. This fact should be considered when addressing TB in human populations.

“Tuberculosis (TB) remains one of the world’s deadliest communicable diseases. In 2013, an estimated 9.0 million people developed TB and 1.5 million died from the disease, 360 000 of whom were HIV-positive. TB is slowly declining each year and it is estimated that 37 million lives were saved between 2000 and 2013 through effective diagnosis and treatment.” (WHO Global Tuberculosis Report 2014).

Factors affecting prevalence of TB in South Africa:

- Immunosuppression due to HIV/AIDS.
- Poor socio-economic factors.
- Emergence of strains that are resistant to drugs used to treat TB (MDR and XDR strains).
- Delay in diagnosis and lost to follow up of newly diagnosed cases.
- Time spent in congregate settings e.g. prisons, gold mines, etc.
- Positive impact of new improved (molecular) tools for TB diagnosis.
- Availability of new anti-mycobacterial agents.

South Africa is facing one of the worst dual epidemics in the world due to concurrent infections with HIV and TB. TB is the most common opportunistic infection and leading cause of death in people living with HIV/AIDS.

Figure 40 - Human TB/HIV statistics for South Africa (WHO)

TB/HIV 2013	Number	(%)
TB patients with known HIV status	294 504	(90)
HIV-positive TB patients	181 736	(62)
HIV-positive TB patients on co-trimoxazole preventive therapy (CPT)	146 973	(81)
HIV-positive TB patients on antiretroviral therapy (ART)	120 298	(66)
HIV-positive people screened for TB	961 967	
HIV-positive people provided with IPT	339 518	

Figure 41 - TB statistics for South Africa 2013 (WHO)

Estimates of MDR-TB burden * 2013	New		Retreatment	
% of TB cases with MDR-TB	1.8%	(1.4–2.3)	6.7%	(5.4–8.2)
MDR-TB cases among notified pulmonary TB cases	4 600	(3 600–5 900)	2 200	(1 800–2 700)

TB case notifications 2013	New **	Relapse
Pulmonary, bacteriologically confirmed	109 630	7 751
Pulmonary, clinically diagnosed	148 658	8 627
Extrapulmonary	37 709	5
Total new and relapse	312 380	
Previously treated, excluding relapses	16 516	
Total cases notified	328 896	

Among 312 380 new and relapse cases:

36 671 (12%) cases aged under 15 years; male:female ratio = 1.2

Epidemiology

Any infected mammal can transmit *M. bovis* zoonotically, but infected cattle are the main source of bovine TB in humans. Modes of transmission include ingestion of unpasteurised milk and milk products, ingestion of infected meat/ organs and inhalation (aerosol) of the bacterium (e.g. when an infected animal coughs or when lesions are cut into during slaughter/ post mortem). Children, immunocompromised people, abattoir workers, AHT's and Veterinarians are at an increased risk of contracting bovine TB.

Several factors affect the distribution and transmission of TB, including overcrowding, social deprivation, population growth, population movements (famine, war, natural disasters, migration, etc.) and occupation.

Transmission

Transmission of human TB is typically caused by airborne spread, usually indoors, via droplet nuclei that stay suspended in the air for hours. Ventilation of such areas will remove droplets and sunlight kills the bacteria. Remember that a source is defined as a smear positive TB person or infected animal.

Two main forms of TB are seen in humans - pulmonary (lung) and extra-pulmonary. The location of the lesions depends on the mode of transmission (inhalation/ ingestion/ cutaneous exposure).

Pulmonary TB in humans is caused by inhalation of infective aerosolized particles. The bacterium establishes itself in the lung parenchyma and tubercles/ granulomas develop. Dissemination may occur via the regional lymph nodes and spread to any part of the body. Organs that may be infected by spread from pulmonary TB - kidneys, liver, spleen, skeleton (children), meninges and reproductive organs. Early treatment can cure lung lesions, but success rates are lower in extra-pulmonary disease, especially TB meningitis. The clinical manifestation of pulmonary TB is mainly seen as a cough. Early in the course of the illness the cough may be nonproductive, but as soon as necrosis starts, sputum is usually produced.

Other clinical signs include weight loss and anorexia, fever and night sweats, chest pain due to irritation of pleural lining, haemoptysis may result from rupture of blood vessel in TB pulmonary cavity and dyspnoea (common in extensive disease).

Whether the infection progresses to clinical disease depends on two factors: The ability of the immune system to control bacterial growth and the virulence of MTB strain (less important than host factors). The clinical manifestations of TB are variable and depend on a number of factors: Age, immune status, specific immunodeficiency states, malnutrition, coexisting diseases and immunization with Bacillus Calmette-Guerin (BCG).

Organs/tissues infected by spread from extra-pulmonary bovine TB include the gastro-intestinal tract/ lymph nodes, mesenterium, joints, skeleton (children), meninges and skin.

Prevention of TB in humans

- Improved socio-economic status
- Basic hygiene measures to prevent transmission
- Cough control and aerosol prevention
- Effective diagnosis (chest radiographs, sputum, culture, molecular testing) and appropriate treatment (MDR, XDR strains)
- BCG immunization of infants (2 days of age) and children



Figure 420 - TB lymphadenopathy



Figure 431 - TB meningitis

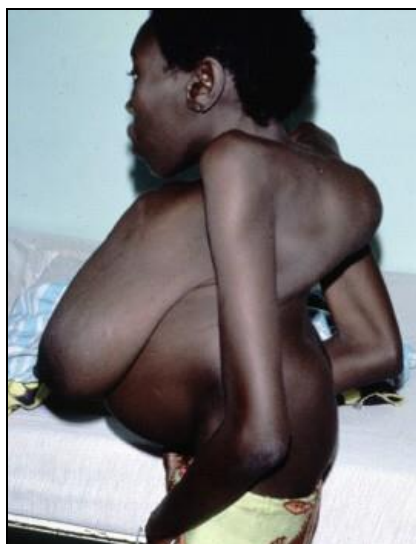


Figure 442 - TB effusions (pleural, pericardial & peritoneal) and spinal TB



Figure 43 - Cutaneous TB lesion

Treatment of Human TB

Curative chemotherapy may be achieved by ensuring that the patient receives at least two drugs to which the causative organism is sensitive (in appropriate dosage) for a minimum of 6 months in case of drug-susceptible TB.

Prevention of spread of infection should be addressed and is achieved by using rapidly sterilizing anti-TB drugs, patient monitoring to ensure the drug regimen is followed, identification and monitoring of close contacts and chemoprophylaxis in certain groups (Children < 5 years, and HIV positive contacts).

Treatment regimens have an initial (intensive) phase followed by a continuation phase. Treatment should be given

not less than five days per week in the intensive phase. It is recommended that treatment should also be given five times per week in the continuation phase, but if that is impossible, then treatment can be given three times per week in the continuation phase only. Note that treatment is intensive and continues for a prolonged period in order to be effective. Patient compliance is crucial in the control of TB. MDR (Multiple Drug Resistant) and XDR (extremely drug Resistant) strains of TB are developing worldwide due to a lack of accurate diagnosis (and drug susceptibility testing) and compliance with treatment regimens.

6 Test Programmes

The execution of the tuberculin tests has as its purpose the detection, control, the combating and the eventual eradication of TB.

It is proposed that only the following programmes be used in future:

- (02) Maintenance herd programme
- (03) Surveillance herd programme
- (04) Diagnostic testing programme (individual animals)
- (09) Infected herd programme

The test programmes (with their computer allocated codes in brackets) which were used in the past are as follows:

- Accreditation (01) – not in use anymore
- Maintenance (old Annual diagnostic) (02)
- Diagnostic herd test (03) – will fall under “Surveillance”
- Ordinary diagnostic (04) – Will fall under “Diagnostic testing programme”
- Import (05) - Will fall under “Diagnostic testing programme ”
- Export (06) - Will fall under “Diagnostic testing programme”
- Isonicotinic acid hydrazide treatment programme – not in use anymore
- Infected (09)

Joining a program is voluntary (except the infected programs). If infection is established at the first or later tests, the herd is accommodated under the infected herd program, and although the owner has joined voluntarily, he cannot withdraw from the programme, thereby avoiding further tests and the slaughtering of positive reactors. Tests and further action for the eradication of the infection is compulsory and can be enforced in terms of the Animal Diseases Act, 1984 (Act No. 35 of 1984).

When a herd has been accepted under any programme, a file is opened for the herd in the State veterinary office. The file number is given in accordance with the system generally in use, i.e. Province number/ local municipality number/ sequential number of herd for that local municipality.

For example: Mpumalanga/Govan Mbeki/1st herd would read as follows: 01/152/01

6.1 Accreditation (01)

When an owner applies to enter the programme the State veterinarian undertakes an inspection to determine whether the farm meets the necessary requirements:

- The farm must be effectively fenced and all neighbouring herds tested at least once in five years
- Public roads running through the farm must be fenced on both sides.
- A suitable quarantine camp with separate water and adequate grazing must be provided in order to isolate positive, or suspect cattle.
- A suitable crush pen and/or other facilities and sufficient aid where animals are tested must be provided.
- Animals on the farm must not use the same grazing, drinking troughs, cow sheds, kraals, crush pen or dip

- The herd must be closed.
- Attention should be paid to hygiene, i.e. the condition of the cows sheds floors, feeding and water troughs, etc. should be of such a nature that they can be thoroughly cleaned and disinfected if necessary.
- Management on the farm is important, because reliable records, indicating all increase and decreases, mutual cooperation and the success of the entire programme depend on it.
- Every animal must be marked in such a manner that individual identification is possible. Identification of cattle is for the owner's personal account.
- Any movement of livestock to the herd is dependent on the approval of the local State veterinarian and the issuing of a transport permit, subject to these conditions.
- Any stillbirths/abortions must be presented for investigation.

6.2 Maintenance (02)

If a herd enters the programme for the first time the agreement (TB 1) is completed in advance and this accompanies the TB 10 together with the tuberculin usage form to the State Veterinarian as well as Deputy Director: Animal Health. **All herds should preferably be incorporated into the maintenance programme.**

It is not a requirement that the skin thickness readings for every animal (normally and after 72 hours) should be entered on TB 10. However, wherever there are cattle with an increase in skin thickness, whether they were diagnosed as negative, suspect or positive, both readings (normal and after 72 hours) shall be recorded with an indication of the nature of the reaction and the diagnosis.

The relevant declaration only states that the animals tested negative for tuberculosis on the specific dates of the test. After a TB/BR3 has been issued, the herd, which includes all animals of all sexes of all owners above the age of 12 months, will thereafter be retested every two years. A TB/BR3 shall be issued after each such negative biennial test.

For each herd a separate file is opened and a reference number allocated as described in the introductory paragraphs of the programmes. The TB 10 is completed in respect of each test.

6.3 Diagnostic Herd (03) - (Herd Surveillance Programme)

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under the Diagnostic Herd Programme. Tests in accordance with this programme should preferably be undertaken on an organized basis e.g. testing the whole municipal area systematically from a certain point until the tuberculosis status of the whole municipality is eventually established. The tests are mainly executed at the State's expense by officials.

Some stock owners who are not prepared to subject their herds to the conditions of the maintenance programme, but are nevertheless anxious to determine the tuberculosis status of their herds can be accommodated under this programme. Tests will be performed at owners' expense. The agreement (TB 1) should be completed in advance and submitted after the test together with the TB 10 to the State Veterinarian for forwarding to the Deputy Director: Animal Health together with the tuberculin usage form.

Separate identification of animals is not required for such tests. However, the owner may identify the animals at his own cost. Cattle that show suspect reactions to the tuberculin test should be identified with ear-tags in order to identify them permanently for further tests.

Should positive reactors be found with the tuberculin test, the herd is incorporated into the "Infected Programme" and, all the animals identified by ear-tags. Skin thickness readings are dealt, with as described in paragraph 6.1.

All cattle of all sexes belonging to all owners on the farm older than 18 months are subjected to the single intradermal tuberculin test. A herd is only tested once under this programme except:

- When suspect reactors are found in which case the whole herd is again tested after three months.
- When the owner wishes to incorporate his herd under the Maintenance Programme. (In such cases the conditions and testing procedures as described under the Maintenance Programme are applicable).

This programme offers an ideal opportunity for stock owners to acquaint themselves in a practical way with the purpose of the tuberculosis tests as well as to identify herds that will later be incorporated into Maintenance Programmes. Stock owners who are, however, satisfied with a single surveillance should nevertheless be advised and motivated to keep their herds free from tuberculosis by means of good management practices, by purchasing cattle from maintenance herds or to have the cattle tested negatively prior to purchasing.

A separate file is not kept for each individual herd tested under this programme except where the test has positive reactors. In such cases the herd is treated further as described under "Infected Programme". Test results of negatively tested herds are placed on one local municipality file in numerical order and the following reference number allocated: Province/Local Municipality/HD. The "'HD'" distinguishes the file from the maintenance and infected herd files. TB 10 and tuberculin usage form are completed and submitted as indicated in paragraph 7.2.6

6.4 Diagnostic (04) - (Diagnostic Testing Programme)

All tuberculin tests, no matter under which programme they are conducted, are in fact diagnostic by nature. All the tests that cannot be incorporated in one of the other programmes mentioned, fall under the diagnostic programme. It includes cattle destined to be exported or imported. Cattle that are imported and are kept at one of the quarantine stations undergo a compulsory tuberculin test. The test, a comparative test with bovine and avian tuberculin (if possible), is undertaken by the officer in charge of the quarantine station.

Cattle that are exported are usually subjected to the comparative tuberculin test at the cattle owner's expense - regardless of whether the test is conducted by a Private- or a State Veterinarian

Such tests are conducted where an owner wishes to test one or more of the cattle in a herd, for e.g. if they show signs suspect of TB. Tests in accordance with this programme are preferably, and where at all possible, executed by private veterinarians at the expense of the stock owner. Where no alternative is available, the test can be conducted by State Veterinarians, but the owner must pay for the test. As the tests conducted in terms of this programme are regarded as a clinical diagnostic service it will only be done by Animal Health Technician's in very exceptional cases.

The agreement (TB 1) should be completed in advance by the stock owner and the Private Veterinarian, and the Bovine Tuberculosis Manual

Approved by DAH:  Date: 2016 -09- 2 0

It is not a requirement that the skin readings of every animal (normal and after 72 hours) should be recorded on TB 10. However, in all cases where there is an increase in the skin thickness, be they diagnosed as negative, suspect or positive, both skin thicknesses, normal and after 72 hours must be recorded with an indication of the nature of the reaction and diagnosis. An individual file is not opened for each herd (except in the case of positive reactors) but all negative diagnostic tests are placed on the same file per municipal area.

Cattle that are imported and are kept at one of the quarantine stations undergo a compulsory tuberculin test. The test, a comparative test with bovine and avian tuberculin, is undertaken by the officer in charge of the quarantine station with a report thereafter on TB 10 and tuberculin usage form a copy of which must be submitted to the Deputy Director: Animal Health. Because the test is compulsory for all imported cattle, no TB1 agreement is completed. These test reports are not placed on a separate file for each owner but in a joint import file as follows per quarantine station

Most importing countries demand that cattle should be subjected to a tuberculin test. To meet this requirement cattle that are exported are usually subjected to the comparative tuberculin test at the cattle owner's expense - regardless of whether the test is conducted by a Private- or a State Veterinarian. Before the test the agreement (TB1) must be completed and the test results forwarded to the Deputy Director: Animal Health together with TB TB10 and tuberculin usage record.

A herd is regarded as infected when infection has been determined as indicated in paragraphs 6.1. to 6.3 during meat inspection, milk examination, post mortem examination and clinical cases, but especially when positive tuberculin tests have been conducted. Such a herd is then placed under quarantine and the necessary steps are taken to eradicate infection in the herd and to keep the herd free from infection thereafter. In the execution of these duties the official is backed by the Animal Diseases Act, 1984 (Act 35 of 1984) and the Regulations enacted under the Act, as well as the Bovine Tuberculosis Scheme regulations.

Meat inspection

Infection found during other investigations

6.7.1 Procedures for handling infected herds

a.) Introduction

Many State Veterinarians and Animal Health Technicians have never had to deal with a TB infected herd. These procedures must be regarded as a guideline only as every herd will differ and must be treated on the merits of that particular case. These guidelines will also aid the farmer's private veterinarian to understand the course of control and to advise the farmer correctly.

b.) Duties of the farmer

- According to the Standing Regulations in terms of the Animal Diseases Act (Act 35 of 1984) the onus rests with the stock owner to keep such animals in quarantine until a State Veterinarian authorizes their release (in cases where a controlled disease mentioned in Table 2 has been determined or is suspected)
- The onus also rests on the owner to inform neighbours and buyers (of milk in this case) if his/her animals are infected or suspected to be infected with a controlled disease.

c.) Duties of the State Veterinarian

- Once TB is confirmed in a herd the State Veterinarian of the area must personally assume complete control over all diagnoses and control measures.
- The State Veterinarian must report the outbreak on SR1 to DAFF.
- The technician may still perform the tests, but the State Veterinarian must be present on the day of reading and must be responsible for the diagnosis of the readings.
- The SV will open a file for the infected farm and all test results and other correspondence must be kept in this file. The file numbering will be as discussed before.
- All contact with the farmer about control measures must be done personally by the State Veterinarian.
- The SV must be present when positive animals are slaughtered.
- The SV must confirm all diagnoses and post mortem results in writing to the farmer as soon as they are available.
- The farmer and his private veterinarian must be kept fully informed of all control measures at all times.

d.) Control over TB positive herd

- As soon as the diagnosis has been made the herd must be put under quarantine with a written quarantine notice.
- A stock register must be opened and full records kept of all increases and decreases. This register must be balanced at every test.
- The farmer must be able to account for every single animal on the farm at all times.
- All conditions in the quarantine notice must be explained fully to the farmer.
- All cattle movements off the farm must take place under cover of a Red Cross permit and in sealed trucks directly to an abattoir or in exceptional circumstances to another farm provided the other farm would also then be placed under quarantine.

e.) Testing procedures

- Depending on the circumstances in the herd either the single or the comparative intradermal test may be used.
- It is important to apply a very strict interpretation in infected herds
- All cattle on the farm must be tested (ie. Beef herd on a dairy farm, labourers' cattle etc.)
- All cattle must have acceptable means of identification (ear tags, brands etc.).
- Positive reactors must be branded on the day of diagnosis
- These reactors must be removed from the herd without delay (the same day if possible)
- If reactors are not removed on the day of testing, then they must be kept separate from all other cattle on the farm.
- In the case of dairy cattle, these animals must be milked last and all facilities cleaned and disinfected after each milking.
- The State Veterinarian will organize the slaughter of the infected animals and the farmer will be responsible for the transport of the animals to the abattoir.

- The technician will be present for the loading of the animals and will ensure that all the reactors are loaded and the truck sealed.
- The technician will then notify the abattoir of the expected time of arrival of the animals.
- A copy of the Red Cross Permit must be forwarded to the SV office.
- Once the reactors have been removed any water and feed troughs, parlours etc. should be thoroughly cleaned out and disinfected. This is particularly important in the case of dairy animals. Disinfectant to be supplied by farmer but disinfection to be done under official supervision.
- Testing must be carried out on a strict three monthly interval.
- Only after 2 completely negative herd tests can the quarantine be lifted. Depending on the circumstances in the herd and the level of management on the farm the State Veterinarian may permit the movement of animals directly to an approved abattoir for immediate slaughter. These movements will be under cover of a red cross permit and in sealed trucks. **NB - Not sufficient in chronically infected herd! It is advisable to continue testing annually for the next 5 years.**

6.7.2 Procedure that must be followed after infection has been determined

a.) Reporting

Infection or suspected infection must be reported to the local State Veterinarian in terms of the Animal Diseases Act, 1984 (Act No 35 of 1984). If a private veterinarian finds positive reactors during a test and there is no Animal Health Technician present to brand the cattle the State Veterinarian is informed telephonically so that arrangements can be made for the cattle to be branded.

b.) Branding of reactors

Positive reactors are, if at all possible branded the same day as the test is read. This is done by or under the supervision of a State Veterinarian, or an Animal Health Technician. Positive reactors are branded with a hot T-branding iron supplied by the State on the left hand side of the neck approximately 15 cm below the junction of the head and neck. Animals that are to be slaughtered within a week or two can be branded lightly but those where postponement for slaughtering has been allowed must be branded thoroughly.

c.) Quarantine

According to the Standing Regulations in terms of the Animal Diseases Act, 1984 (Act 35 of 1984) the onus rests with the stock owner to keep such animals in quarantine until a State Veterinarian authorizes their release in cases where a controlled disease mentioned in Table 2 has been determined or is suspected. The issuing of a written quarantine notice is therefore not required. However, to obviate any doubt also as far as later court cases are concerned it is usual to issue a written notice in the prescribed manner where bovine tuberculosis has been determined.

d.) Permits

A permit is necessary for moving cattle from, to and through the farm. A red cross permit is required for any animal movement off the farm (irrespective of test result) and movement is only allowed to an abattoir.

Salvage procedure/ subdivision of herd: If a farmer wishes to salvage herd members through subdivision of a herd, there needs to be an application to the DAH (Director Animal Health) through the local State Veterinarian and Provincial Director to move the test negative herd members to a “clean” farm. The farm has to be fenced, adequate biosecurity and management procedures need to be in place and all movements controlled through red cross permits. Testing will continue for a satisfactory period (depends on herd dynamics and structure) until the herd can be declared negative.

e.) Arrangement regarding reactors

In terms of existing legislation and the present scheme we are compelled to deal with reactors in such a way that

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the danger of further spreading of the disease is eliminated as far as possible and that infection will eventually be eradicated in the herd.

One of the following can be applied:

- Immediate slaughtering
- Trial slaughtering
- Postponed slaughtering

i) Immediate slaughtering

Animals can be slaughtered according to law to prevent the spreading of the disease and to eradicate it. The owner may apply for compensation which, at present, would be the slaughter value that he would have been paid at the abattoir should the animal be condemned. The State Veterinarian must be present at slaughter, or if slaughtered in another State Veterinarian area, arrange that a State Veterinarian is present at slaughter so as to ensure proper meat inspection. If the carcass is condemned, then a statement from the abattoir as to what the owner would have been paid should the carcass have been passed must be obtained, so that the amount of compensation can be determined.

ii) Trial slaughtering

During the first test on a herd the veterinarian might be doubtful whether infection is in fact present in the herd. In this case is it desirable to select two or three cattle for a trial slaughtering.

Selecting animals for trial slaughtering - Young animals with large and typical reactions are **not** suitable for this purpose. Such animals may be in an early stage of infection and thus not have macroscopically observable lesions. It is advisable to select old animals with reasonably typical reactions or animals older than 5 years with a fair degree of skin thickening. If the older animals in addition also show clinically suspect signs of disease, they are the ideal candidates to select for a trial slaughtering.

iii) Postponement of slaughtering

Under certain conditions the immediate slaughtering of a large number of reactors may seriously affect the owner financially, disrupt his farming business or even hamper the provision of milk to a community.

Postponement of slaughtering is considered when:

- animals of a good quality such as high milk producers are involved;
- the percentage of animals infected is high, for example 20% to 30%;
- a large number of positive animals are found (majority of the herd);
- there is a specific reason for postponement in respect of specific animals such as cows at the top of their lactation or cows with small calves at foot.

If slaughtering is postponed:

- Separation - Postponement can only be granted when the positive animals are separated in such a way that they pose no danger for susceptible uninfected animals.
- Duration of period - After the beneficial lactation period has passed or a calf has been weaned, the cow must be slaughtered. The period will therefore not exceed twelve months. The purpose of this postponed slaughtering is to enable the owner to make alternate arrangements and not to keep on farming indefinitely with an infected herd.
- Milk for human consumption - Milk coming from positive reactors must be boiled, pasteurised or sterilized before it can be used or made available for human or animal consumption or before it can be sold. Cows that produce infected milk should be slaughtered without delay.
- Clinical cases - Advanced clinical cases or reactors that are old and so emaciated that there is little doubt that the animals will be generally rejected as a result of tuberculosis may, if the State Veterinarian so decides, be slaughtered on the farm, thoroughly examined and destroyed. In this way a source of infection is immediately removed and cost of transport to the abattoir saved.

f.) Disinfection

The disinfectant that is normally used in cowsheds is 3% formalin whilst 3% wescodyne or F10 is used for milking equipment.

Formalin – 3%. When 1 part 40% formaldehyde is mixed with 12.5 parts water, it will give a 3% disinfectant.

Surfaces that are disinfected should be kept wet for at least 20 minutes with the disinfectant.

- Phenol & Cresol – 3% for 20 minutes.
- Sodium hypochlorite (NaOCl) – 2% for 20 minutes
- Calcium hypochloride ($\text{Ca}(\text{OCl})_2$) – active chloride should be at least 30% for 20 minutes
- Chloroxylenol – 2% for 20 minutes.
- Lime (CaO) – 20% can be used to whitewash walls

Caustic soda (NaOH) does not kill tuberculosis bacteria and is therefore not recommended.

The disinfectant must be supplied by the stock owner where infection has been found whilst the State Veterinarian prescribes the type of disinfectant and the method of disinfection.

The process must take place under official supervision and takes place just after the positive cattle have been removed from the herd. i.e. have been sent to the abattoir or have been placed in the quarantine camp. Everything in the cowshed as well as feeding and water troughs outside such buildings which could possibly have been infected by the cattle, must be disinfected. All manure, hay and other refuse are first removed from the building to a place out of reach of susceptible animals. Thereafter it is washed with water where possible. After cleaning, the disinfectant is applied according to prescription.

The disinfecting process is repeated 14 days later.

If new positive cases are found on retesting, the disinfecting procedure is repeated

g.) Tests

In a herd with a considerable number of positive reactors it is strongly recommended that the second test be done six weeks later for the sake of re-sensitizing possible anergic cases. The second test should in any case be conducted not later than three months after the first. Thereafter further tests are conducted at three monthly intervals as long as new positive cases are found.

- After two negative tests on the herd, movements to other herds may take place after the quarantine has been lifted. However, the herds remain under official supervision and are incorporated into the maintenance scheme to prevent re-infection of the herd or to trace infection in time before it again becomes prevalent. In this way we want to remain in control of conquered areas. **Such previous infected herds should be retested annually for 5 years.**

6.7.3 Files

Irrespective of the purpose for which the first test was done. A separate file must be opened for each herd where infection has been found. A file number is allocated according to the customary method as described before,

7 Administration of the Scheme

7.1 Forms in use (available from local State Veterinarian)

7.1.1 Application for TB/ BR testing (TB/BR 1)

This form is used as an application by the stock owner to comply with the requirements for admittance to any of the test programmes.

The use of the form is an undertaking by the stock owner. The agreement is a legal document and should be

completed fully (also date and place) and be signed by the stock owner or his authorized representative before a test is conducted on a herd for the first time. The application, together with the first test reports, is completed in Triplicate. The original is sent to the State veterinarian and a copy to the owner.

7.1.2 TB/ BR test declaration (TB/BR3)

(Refer also to the Bovine Tuberculosis Scheme Regulations.)

A declaration by means of a TB/CA 3 issued to any negative maintenance herd that has been tested as below and is issued by a State veterinarian.

Maintenance programme: after the second negative test and thereafter biennially following the TB test on the herd.

Infected programme: issued after the second negative test, thereafter annually for 5 years & thereafter biennially following the negative TB test on the herd.

7.1.3 Monthly Statement of tuberculin tests (TB 7)

a.) Office of the State Veterinarian

Here provision is made for a summary of each test conducted during the month for which the State Veterinarian has received a report which is forwarded to the Deputy Director: Animal Health of the District.

b.) Office of the District Deputy Director: Animal Health

Here a composite summary for the whole district is drawn up & submitted to the Director: Animal Health of the province.

c.) Office of the Provincial Director: Animal Health

Here a composite summary for the whole province is drawn up & submitted to the National Director: Animal Health.

7.1.4 Abattoir findings

The notification of bovine tuberculosis found at an abattoir takes place telephonically and also per the meat inspection report form in terms of the Meat Safety Act, 2000 (Act No. 40 of 2000) as indicated under 'meat inspection' in this manual. The State Veterinarian or Meat Inspector where the cattle are slaughtered, completes the form because the infection comes to his attention or should come to his attention and because it is also easier for him to get the information regarding the farm of origin of the animal/s. Meat inspection at abattoirs is an important aid for the tracing of infected herds provided that everything possible is done to get the information without delay from the abattoir and thereafter determining the herd's origin if it is at all possible. The form is then sent to the State Veterinarian at the place of origin of the animal/s with copies to the relevant Deputy Director: Animal Health. A quarantine notice is issued against the owner and the whole herd is tested as soon as is feasible. After the Deputy Director: Animal Health has received a copy of the TB report it should be kept in abeyance for one month. If after the expiry of a month no report of the examination and test results has been received, enquiries can be made at the office of the State Veterinarian thereby ensuring that the herd has not been forgotten.

Condemnations

- The entire carcass and all the organs must be condemned where there is:
 - TB associated with fever and/or emaciation
 - Evidence of widespread lesions (more than one organ affected or milliary lesions in one organ)
 - TB in inter-muscular lymph nodes, liver, muscular tissue, bone, joints or central nervous system
 - Any acute extensive exudative TB of pleura, peritoneum, pericardium or meninges
- When the disease is localized, the associated organ and lymph nodes must be removed and condemned; and the rest of the carcass passed

7.1.5 Intradermal tuberculin test record (TB10)

This form is completed by the testing official or veterinarian in respect of all cattle tested & must be submitted as soon as possible, but no later than 30 days after having performed the test (together with a copy of the tuberculin usage form for both avian & bovine tuberculin as applicable), to the State Veterinarian of the area, (in terms of Section 8 (2) of the bovine Tuberculosis scheme regulations) who must check it, sign it & forward a copy of the signed document to the Deputy Director: Animal Health.

The top portion, in respect of the details of the owner & farm are always to be completed in full. In respect of tests at communal dip tanks the owner could be reported as "various".

- The test from date = date of injection of tuberculin
- The test date to = date of reading the tuberculin test (which **must** always be 3 (three) days later)

The second portion of the form must always be completed as fully as possible.

- 1st line – **Printed** initials & surname of tester
- 2nd line – previous test date & previous number of animals tested – to be completed if known. This may have to be completed in the State Veterinarian office if not completed by the tester (as tester may not have such records but State Veterinarian office should have records if herd previously tested)
- 3rd line:
 - No. of animals on farm – ask farmer & always complete
 - No. injected – number of animals actually injected with tuberculin – **keep accurate record and complete in all cases**
 - No. read – actual number of animals presented for reading of TB test on day 3. Should be same as number tested but in some cases (i.e. communal areas, animal died, etc.) it will not be & thus **keep accurate record of those read and complete in all cases**
- 4th line – Result:
 - Number Neg – total of animals diagnosed as negative i.e. those measured because of skin reactions but diagnosed as negative **as well as** those which have been felt/read but which showed absolutely no reaction at tuberculin injection site (in most cases these will be most of the animals in a herd)
 - Number Pos – total of animals diagnosed as positive. Obviously this **must** agree with the animals diagnosed and recorded as positive in 3rd portion of form
 - Number Susp – total of animals diagnosed as suspicious. Obviously this **must** agree with the animals diagnosed and recorded as suspicious in 3rd portion of form
 - Total Read = Number Neg + Number Pos + Number Susp. This must obviously agree with the Number Read in line 3 of this section
- 5th line:
 - Cattle breed – always complete. Ask farmer if not sure. Cattle will always have some breed characteristic i.e. not just mixed beef – rather Brahman X etc. Please ensure if doing TB and CA test on herd that the cattle breed mentioned on CA8 and TB10 agree for same farm and test dates!
 - Delivers milk to: This obviously only to be completed in case of dairy herds i.e. whom is milk buyer if sells to a distributor or of sells locally then mention where
 - Herd test type: Mark what is applicable. Remember that a herd will always be regarded as an infected herd as soon as there are positive reactors, no matter what the first reason for testing the herd was.
- 6th line:
 - Signed: Signature of tester
 - Date: Date signed by tester. Should be the same day or at most the day after reading the test.
 - Contact no of tester: Tester's cell phone number

The third portion of the form is however completed differently for the various programmes.

The fourth portion of the form is completed and signed by the State Veterinarian of the area after checking the form for correctness and whether he/she agrees with the diagnosis of each animal. A copy must then be sent to the Deputy Director: Animal Health.

The first two portions of the form are completed in full as described above.

- No allotted to animal:
- 1.....etc. The allotted number in case of maintenance and infected herds is always the same for the same animal. Normally would write all animal numbers down and then when back at the office - organize the ear tags in description column in alphabetical, numerical order along with the allocated allotted number; otherwise record keeping at next test becomes very difficult.
- The number allotted to an animal during the 1st test is kept for all subsequent tests and the number is NOT given to another animal
- Description of each animal tested
- Ear tag, tattoo or individual animal brand number
- Breed of animal – if crossbreeds the describe the colour of the animal
- Sex: cow/heifer, ox, bull
- Age: approximate age of animal – very NB for positive reactors
- When doing the test it is not necessary to measure each animal at time of testing but at least mark that is present. Again when reading the test three days later every animal's injection site must be palpated, and the animal marked off as present, but only those with palpable reactions will be measured & recorded – in the cases of animals showing palpable/visible reactions the measurements are noted as follows:
 - Bovine tuberculin injection site:
 - Normal Bovine skin fold: Measure skin above the bovine injection site (if comparative test was performed this will be the rear injection site)
 - 72hrs Bovine skin fold: Measure the skin across the thickest part of the swelling – in cases where there is oedema the swelling may sag down
 - Bovine difference = Swelling – normal
 - Avian tuberculin injection site:
 - Normal Avian skin fold: :Measure skin above the avian injection site (if comparative test was performed this will be the front injection site)
 - 72hrs Avian skin fold: Measure the skin across the thickest part of the swelling – in cases where there is oedema the swelling may sag down.
 - Avian difference = Swelling – normal
 - Bovine diff – Avian diff. If the bovine swelling is larger this will be written as (+), if the avian swelling is larger this will be written as (–).
 - Remarks: Describe only the bovine reaction site, as well as the enlarged prescapular lymph nodes (if applicable). Use the shorthand as described earlier.
 - Diagnosis: The diagnosis per animal must be written here, i.e. Pos (Positive), Susp (Suspicious) or Neg (Negative).

The first two portions of the form are completed in full as described above.

7.1.6 Instruction (order form (TB/BR11))

This form is only used in exceptional cases, for example if the herds in an area or district are being tested systematically under the diagnostic herd programme and some owners who refuse to fall in line, can be compelled to test. This should be issued by the State Veterinarian personally after discussion with the Deputy Director: Animal Health.

8 Tuberculosis – Legislation

The Animal Diseases Act, 1984 (Act 35 of 1984), Animal Diseases Regulations (R2026 of 26 September 1986) and the Bovine Tuberculosis Scheme Regulations (R1953 of 30 September 1988), all as amended, give the necessary authorization for the control, combating and eradication of bovine tuberculosis.

The measures for combating tuberculosis can be summarized as follows, mention being made of the relevant Sections of the Act and Regulations applicable: *(with credit to Dr Ben du Plessis for the tables below)*

ACT 35/1984 SECTION	ANIMAL DISEASES REGULATIONS	SUBJECT	ASPECTS w.r.t. TUBERCULOSIS
9 (2) (a) 11 (1) (b) (i) 31	11, table 2	Control measures Duties of owners/managers	Testing Isolation Marking Slaughtering Treatment
9 (2) (h) 11 (1) (b) (ii) 31	12	Reporting Duties of owners/managers	Reporting of incidence or suspected incidence of controlled disease by responsible person/veterinarian to State Veterinarian/Animal Health Technician
9 (2) © 11 (1) (a) 31	13	Isolation Duties of owners/managers	Isolation of contact or infected animals
9 (2) (d) 11 (1) (a) 31	14	Prohibition of access Duties of owners/managers	Prohibition of access to places with isolated animals
9 (2) (a) 11 (1) (a) 31	15	Disinfection Duties of owners/managers	Timing, effectivity, concentration, extent, removal and disposal with regard to disinfection of places, conveyances and appliances; inaccessibility of places to animals; washing of person, clothes and equipment.
9 (2) (h) 11 (1) (b) 31	16	Sampling Duties of owners/managers	Taking, preservation, treatment, packing, dispatching and delivery of samples.
9 (2) (b) 31	18	Proof of performance of controlled veterinary acts	Certificate, document, sworn declaration, containers and invoices pertaining to controlled veterinary acts performed in terms of regulation 11.
9 (2) © 31	20 (1) (a) (xiii)	Movement restrictions	Prohibition of movement to accredited herds
9 (2) © 31	22	Slaughter restrictions	Prohibition of slaughter of isolated animals
9 (2) (a) 31	24 (1) (a)	Disposal restrictions	Prohibition of use and disposal of unboiled, unpasteurised or unsterilised milk from infected or suspected infected animals
10		Animal health schemes	See below
15 31	28	Orders	Serving, binding, authority, amending, proof of orders
16		Powers of entry and inspection	Entry upon land and conveyances, assistance, searching, investigation, inspection, marking, testing, interrogation
16 (2) (a) (xiii) 31	29, table 3	Marking	Indication of infection by "T" branding on the left side of neck
19 31	30	Compensation	Extent of compensation for infected and killed animals and infectious or contaminated things
25		Secrecy	Prohibition of disclosing of and access to information, exceptions

Bovine Tuberculosis Scheme

(R1953 of 30 September 1988) established under section 10 of Act 35 of 1984

SECTION	SUBJECT	BRIEF DESCRIPTION
1	Definitions	Meaning of word and expressions
2	Name of scheme	Bovine Tuberculosis Scheme
3	Object of scheme	(1) Eradication of bovine tuberculosis (2) by testing, identification, slaughtering, isolation, prevention of contact and information.
4	Application and scope of scheme	(1) <i>Mycobacterium bovis</i> , cattle (2) Objects of six programs; accredited herd*, annual diagnostic (maintenance), diagnostic herd, infected herd, isonicotinic acid hydrazide** treatment and diagnostic testing.
5	Manner of infection	(1) Secretion and excretion of <i>Mycobacterium bovis</i> (2) Infection by inhalation, licking, grazing or consuming fodder, water or milk.
6	Characteristics of infection	(1) Mostly no symptoms (2) Advanced stage symptoms (3) Post mortal exhibition of tubercles
7	Tests for bovine tuberculosis	(1) Tuberculin test (2) Interpretation (3) Other tests
8	Requirements relating to a tuberculin test	(1) Only by an officer, authorized person or veterinarian (2) Notification to State Veterinarian of results (3) Prohibition of removal of cattle during test (4) Making cattle available for testing
9	Notification of infection	Written notification of State Veterinarian of infection or suspected infection
10	Measures applying to infected herds	(1) State Veterinarian to order isolation of infected cattle herd or suspect cattle (2) Prohibition of movement of cattle on land with isolated cattle or cattle herds (3) Authorization to move cattle on land with isolated cattle or cattle herds (4) Identification of isolated cattle (5) Record keeping of isolated cattle (as in regulation 17) (6) "T" brand on left side of neck, separation, prohibition on retesting and authorization of retesting of infected cattle (7) Retesting of suspect cattle (8) Retesting of infected cattle herd
11	Revocation of isolation	(1) Conditions for revoking isolation order of infected cattle herds (2) Conditions for revoking isolation order of suspect cattle
12	Disinfection of certain places and things	Manner, frequency and remedy for disinfection of structures on land with infected cattle herd (as in Regulation 15)
13	Disposal of infected bovines	(1) Slaughtering of infected cattle at an abattoir or on land (2) For account of responsible person (3) Meat inspection by veterinarian (4) Compensation (as in section 19 of Act 35 of 1984) (5) Time of slaughtering
14	Application of INH treatment**	(1) Prohibition of INH treatment (2) Permission of INH treatment (3) Agreement, undertaking to comply
15	Requirements for joining scheme	(1) – (6) Requirements for joining each program (see table below)
16	Admission to scheme	(1) Application by responsible person to participate in accredited

		herd*, annual diagnostic (maintenance) herd, diagnostic herd or diagnostic testing programs (2) Admittance of responsible person to infected herd or isonicotinic acid hydrazide treatment programs**
17	Refusal of applications	Refusal of application due to non-compliance with requirements or inability to render services
18	Register of responsible persons and herds	State Veterinarian to keep a register with particulars of admitted responsible persons
19	Lapsing and cancellation of participation	(1) Conditions for lapsing (2) Conditions for cancellation (3) Additional conditions for lapsing or cancellation of participation in infected herd and INH treatment programs**
20	Switching from one program to another	Conditions for switching from one program to another
21	Measures relating to participating herd	Measures pertaining to contact, sharing, introduction of cattle to herd, making cattle available for testing, applying control measures, recordkeeping and marking
22	Issue of certificates and declaration	(1) Issuing and duration of validity of certificate i.r.o. cattle herds in accredited program* (2) Issuing of declaration i.r.o. cattle herds in annual diagnostic (maintenance) or diagnostic herd programs (3) Issuing of declaration i.r.o. cattle herds in infected herd or INH programs** (4) Issuing of declaration i.r.o. cattle in diagnostic testing program (5) contents of declaration
23	Renewal of certificates	(1) Application for renewal of certificate (2) Tuberculin testing (3) Conditions for renewal of certificate (4) Duration of validity of certificate
24	Lapsing of certificates	(1) Conditions for lapsing (2) Conditions for issuing of new certificates in case of lapsing
25	Return of certificates	Return of lapsed certificates
26	Restrictions on the use of certificates and declarations	(1) Prohibition of misuse of declarations (2) Conditions for use of certificate
27	Tariffs for services rendered	(1) Fees for services rendered as in regulation 27 (2) Free services (3) Disinfectants and remedies at expense of responsible person (4) INH** supplied free of charge, tuberculin supplied free of charge for infected or suspected cattle herd
28	Commencement of scheme	1988-10-01

*Accredited Scheme not in use anymore

** Isonicotinic acid hydrazide treatment not in use anymore

Requirements for joining the Bovine Tuberculosis Scheme

(Section 15 of Bovine Tuberculosis Scheme)

Requirement Program	Annual Diagnostic (Maintenance)	Diagnostic Herd	Infected Herd	Diagnostic Testing
Closed herd management	X		X	
Fencing			X	
Isolation facilities			X	
Handling facilities	X	X	X	X
Written undertaking to co-operate	X	X	X	

9 Animal Disease reporting codes:

The Disease reporting codes (Province Code, State Vet Code and Local Municipality Code) contained in the Disease Reporting Manual should be used for the TB Scheme.

A copy of the Disease Reporting Manual can be downloaded from www.daff.gov.za
Branches>>Agricultural Production, Health and Food Safety > Animal Health > Epidemiology
OR

Send an email to epidemiology@daff.gov.za requesting a copy of the manual.