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Regulation

From the Ministry of Agriculture and Rural Affairs:

**REGULATION ON BOVINE TUBERCULOSIS**

**CHAPTER ONE**

**Objective, Scope and Definitions**

**Objective**

**ARTICLE 1** – (1) The objective of this Regulation is to lay down relevant principles and procedures to control and eradicate Bovine Tuberculosis, as well as establishing the rules to achieve “Tuberculosis-free Herd” status.

**Scope**

**ARTICLE 2** – (1) This Regulation shall apply to provisions on the protection of bovine and other domestic animals from tuberculosis.

**Legal basis**

**ARTICLE 3** – (1) The Regulation hereby has been drafted;

a) On the basis of Law No. 3285 of 8/5/1986 on Animal Health and Control,

b) and in line with European Union Council Directive 64/432/EEC on “Animal Health Problems Affecting Intra-Community Trade of Bovine Animals and Swine”, and Council Directive 78/52/EEC on “The Community Criteria for National Plans for the Accelerated Eradication of Brucellosis, Tuberculosis and Enzoitic Leucosis in Cattle”.

**Definitions**

**ARTICLE 4** – (1) For the purposes of this Law;

a) Minister means the Minister of Agriculture and Rural Affairs,

b) Ministry means the Ministry of Agriculture and Rural Affairs,

c) Region means any part of the country, which is at least 2000 km<sup>2</sup> large, subject to the inspection of authorized unit and includes at least one province,

ç) Heat treatment means a treatment, which leads to negative reaction in the alkaline phosphatase test immediately after any application including heating, except boiling.

d) Provincial directorate means the provincial directorate of the Ministry of Agriculture and Rural Affairs,

e) Means of transport mean parts of motor vehicles, railroad vehicles and aircrafts designated for loading, lockers of ships and containers used in road, rail or air transportation,

f) Officially tuberculosis-free zone means a region that complies with the conditions referred in article 15,

g) Officially tuberculosis-free cattle herd means a cattle herd that fulfills the conditions referred in articles 12 and 13,

ğ) Official veterinarian means a veterinarian employed under central or local organizations of the Ministry of Agriculture and Rural Affairs,

h) Bovine animal means cattle and ox of all ages, including Bison bison and Bubalus bubalus but except animals raised for cultural and sporting activities, which are raised for meat production, milk production, for breeding or other purposes,

ı) Herd means an animal or a group of animals that forms an epidemiological unit, and in case more than one animal groups are present at a holding, it means that each herd would comprise a different unit and possesses the same health status,

ı) Suspicious animal means bovine animals that neither the disease could be confirmed nor the possibility of disease could be ignored, and show the symptoms that indicate the potential presence of tuberculosis,

j) Collection center means holdings, collection centers or markets certified by the Ministry, where bovine animals originating from different holdings are gathered in order to form groups to be consigned for commercial purposes,

k) Cattle herds of Type T1 mean herds, clinical history and tuberculin test results of which are unknown,

l) Cattle herds of Type T2 mean herds, clinical history and tuberculin test results of which are known, and routine monitoring tests are applied in conformity with national rules so as to achieve herd of type T3 status,

m) Cattle herds of Type T3 mean officially tuberculosis-free herds,

n) Authorized veterinarian means a veterinarian authorized by the Ministry of Agriculture and Rural Affairs, and working under the authority and responsibility of an official veterinarian,

o) Authorized unit means the provincial or county directorate of the Ministry of Agriculture and Rural Affairs.

## **CHAPTER TWO**

### **General and Special Provisions, Disease Detection, Infected Areas, Procedures to be Followed after Slaughter and Transportation of Animals**

## **General provisions**

**ARTICLE 5** – (1) The Ministry shall draw out a program for the eradication of tuberculosis in bovine animals. The measures to be taken in order to complete the mentioned eradication program successfully are specified below.

a) The proportion of the national cattle population included within the scope of eradication and preventive measures shall be increased every year until the whole population is included.

b) As soon as the official veterinarian completes the necessary procedure, the compensation for the animals culled due to Bovine Tuberculosis shall be paid to breeders in the shortest time.

c) The Ministry shall specify a national reference laboratory for Bovine Tuberculosis and provide sufficient conditions and laboratory staff required for carrying out reference laboratory diagnoses.

ç) Authorized unit shall draw out official records, which classify cattle herds included in the eradication program according to their state of health, and keep these records updated.

d) Provided that an animal suspected to have bovine tuberculosis is detected in a herd, in order to determine whether the disease is present or not, the authorized unit shall enable official inspections to be performed as soon as possible. Tuberculin reaction charts for tuberculin tests applied by the official veterinarian or the authorized veterinarian under the supervision of the official veterinarian shall be drawn up and confirmed by the Provincial Directorate.

## **Special provisions**

**ARTICLE 6** – (1) The presence or the suspicion about the presence of bovine tuberculosis shall be notified to the authorized unit immediately.

(2) Curing bovine tuberculosis or treating the sensitivity, and applying vaccinations against bovine tuberculosis are prohibited.

## **Detecting animals suspicious to have bovine tuberculosis**

**ARTICLE 7** – (1) In case an animal suspicious to have bovine tuberculosis is detected in a herd, the authorized unit shall enable official inspections to be launched as soon as possible in order to determine whether or not the disease exists.

(2) Until inspections are concluded, the authorized unit shall keep the herd under surveillance.

(3) Admittance or exit of animals to the herd shall be prohibited except for the cases when the authorized unit allows dispatch for slaughtering.

(4) The suspicious animals in the herd shall be isolated.

(5) Practices referred in this article shall be continued until it is officially detected that the existence or the suspicion of bovine tuberculosis in the herd is prevented.

## **The official detection of the bovine tuberculosis**

**ARTICLE 8** – (1) Provided that bovine tuberculosis in the herd is officially detected, the authorized unit shall take necessary measures to prevent the spread of the disease, and enable that the following procedures are carried out;

a) All animals in the herd, which includes the infected animal shall be examined for bovine tuberculosis. As regards tuberculin tests applied in the herd by the official veterinarian or the authorized veterinarian under the supervision of the official veterinarian, tuberculin reaction charts shall be drawn up and approved by the Provincial Directorate. In tuberculosis, quarantine is limited to the barn or the herd in which the disease is observed. When tuberculosis is detected, the Animal Health Control Commission shall meet. The decision on the presence of the disease shall be made and announced on the basis of the disease report drawn up by the official veterinarian. The quarantine imposed for tuberculosis shall be maintained until all cattle older than six weeks have negative results in the last two official tuberculin tests. The first test shall be done at least sixty days after the positive reactor is separated from the herd, and the second test shall be done at least four months and at most twelve months later. There should be at least sixty days between the first and second tests.

b) Admittance or exit of animals to the herd shall be prohibited unless the authorized unit allows dispatch for slaughtering.

c) Cattle officially detected to have bovine tuberculosis shall be isolated within the herd.

ç) Cattle officially detected to have bovine tuberculosis shall be isolated until slaughtering and marked. Procedures referred in the relevant legislation shall be applied about the carcasses of the slaughtered cattle.

d) Milk from infected cows may be used for the feeding of the animals in the same farm after treated with appropriate heat treatment, but cannot be used as human food.

e) Milk from cows in an infected herd cannot be transported to milk processing holdings except for processing with appropriate heat treatment, or put on sale as raw milk.

f) Carcasses, half carcasses, limbs or legs, parts and offal of infected cattle, which are going to be used as animal feed, shall be processed in such a way to avoid contamination.

g) Materials that are produced with regard to official arrangements about controls such as waste safety shall be ensured not to have the risk of spreading tuberculosis in any way.

ğ) Manure achieved from the compartments where infected animals are present shall be kept at a place out of the reach of other farm animals, treated with an appropriate disinfectant and kept aside for at least three weeks. In case these manures are covered by uninfected manure or soil, the use of disinfectants shall not be necessary. If liquid wastes from the compartments used by infected animals are not collected along with manures, they shall be disinfected as well.

(2) Infected cattle detected by the authorized unit to have tuberculosis shall be slaughtered under official supervision in the shortest time, on condition that this period would not exceed thirty days.

(3) The owner of animals or the person put in charge on his behalf shall be officially informed about the test results for bovine tuberculosis and his obligations.

(4) However, if animals, which do not show any clinical signs but which are officially detected to have tuberculosis, are found in a holding, the thirty-day slaughtering period can be expanded in the following conditions provided that it does not exceed three months.

a) If there are animals expected to give birth in three months,

b) It is not possible to carry out the slaughtering in thirty days due to technical reasons related with the capacity of the slaughterhouse.

#### **Areas infected with tuberculosis**

**ARTICLE 9** – (1) The authorized unit shall ensure the following procedures to be carried out in relation to infected areas;

a) After the slaughter of infected animals and before the inclusion of new animals to the herd, the barns, other compartments, all containers, equipments and other devices used by the animals shall be cleaned and disinfected under the supervision of an official veterinarian according to the specified instructions.

b) After the transport of infected animals, materials achieved from these animals or materials and substances that have been in contact with these animals, all means of transportation, containers and equipment shall be cleaned and disinfected. After use, loading places of these animals shall be cleaned and disinfected with disinfectants such as 5% phenol, highly concentrated iodine solution, glutaraldehyde and formaldehyde, 1% sodium hypochloride etc.

c) Materials to be used for disinfection shall be certified by the Ministry.

#### **After the slaughtering process of infected cattle**

**ARTICLE 10** – (1) The authorized unit shall ensure the following after the slaughtering process of infected cattle;

a) None of the cattle shall be allowed to be released from the herd in question, except infected animals allowed by the authorized unit for dispatch to slaughtering.

b) In order to detect that the disease is eradicated from the herd, tuberculin tests shall be applied on the herd in question.

c) As regards tuberculin tests applied in the herd by the official veterinarian or the authorized veterinarian under the supervision of the official veterinarian, tuberculin reaction charts shall be drawn up and approved by the Provincial Directorate.

ç) The performance of tuberculin tests on cattle in the infected herd older than six weeks for one or more times shall be ensured, and until it is detected that there is no disease in the herd, no animal from outside shall be allowed to enter the herd.

(2) Within the scope of tuberculosis eradication program, the authorized unit shall ensure all cattle older than six weeks to be put under official surveillance in type-T1 and T2 herds, and assure these cattle to be subject to intradermal tuberculin test at least once in every six months. These tests shall be done until the herd achieves type-T3 herd status.

### **Transportation of animals**

**ARTICLE 11** – (1) During transportation of animals from type-T1 herd to type-T2 herd, the authorized unit shall assure the following:

a) The performance of intradermal tuberculin test on the animal within the thirty days before its transport shall be ensured, and that the document submitted by the official veterinarian, which proves that this operation is carried out, shall be kept during transportation.

b) Immediately after the arrival of the animal to the herd, its isolation shall be assured; the isolation shall be maintained for sixty days and the animal shall be subjected to intradermal tuberculin test before its admittance to the herd.

(2) During transportation of animals from type-T2 herd to another type-T2 herd, the authorized unit shall assure the following:

a) The performance of intradermal tuberculin test on the animal within the thirty days before its transport shall be ensured, and that the document submitted by the official veterinarian, which proves that this operation is carried out, shall be kept during transportation.

b) It shall be ensured that the animal does not contact with any other cattle from a herd of lower health status during transportation.

(3) In all transportations between type-T3 herds, the authorized unit shall assure that the cattle do not contact with other cattle from a herd of lower health status.

(4) Furthermore, the authorized unit shall,

a) Take the official control measures to prevent re-infection of a herd in which tuberculosis is eradicated from other sources of infection,

b) Ensure that all animal transports within the herd within the scope of the eradication program and between herds are subject to official inspection,

c) Ensure that the measures about the control of animal transports are also applied to officially tuberculosis-free herds and to animal transports from these herds.

## **CHAPTER THREE**

### **Officially Tuberculosis-Free Herds, Persistence of the Tuberculosis-Free Status, Suspension, Annulment, and Monitoring and Control of the Status, the Announcement of Tuberculosis-Free Status**

#### **Tuberculosis-free cattle herd**

**ARTICLE 12** – (1) A cattle herd is considered tuberculosis-free when the following conditions are fulfilled:

a) None of the animals show clinical signs of bovine tuberculosis.

b) All cattle older than six weeks show negative reaction to tuberculin tests conducted in compliance with the criteria laid down in chapter four. The first test shall be performed six months after the elimination of tuberculosis from the herd, and the second test shall be done in the following six months. If infection is not detected in the first test performed on the herd and the entire herd show negative reaction in the second test done six months later, the herd shall be given tuberculosis-free status by the authorized unit. However, if the herd consists of animals gathered from officially tuberculosis-free herds, the first test shall be done at least sixty days after the gathering of the herd while the second test need not be performed, and tuberculosis-free status shall be granted by the authorized unit.

(2) After the performance of the first intradermal tuberculin test to the herd, animals older than six weeks shall not be allowed to enter the herd except animals on which intradermal tuberculin test is carried out in accordance with the criteria laid down in chapter four thirty days before or thirty days after the arrival to the herd and which show negative reaction to the test. If the test is performed thirty days after the arrival of the animal to the herd, the animal shall be isolated in such a way to prevent the direct or indirect contact of the animal with others until negative test result is obtained. Yet, if animals are coming from officially tuberculosis-free herds, the authorized unit may decide not to perform the test for these.

#### **Maintenance of the tuberculosis-free status**

**ARTICLE 13** – (1) A cattle herd shall maintain its official tuberculosis-free status in case of the following conditions;

a) Maintenance of the conditions laid down in subparagraph (a) of the first paragraph and the second paragraph of Article 12.

b) Arrival of all animals included in the holding from herds having the tuberculosis-free status.

c) Except for the animals younger than six weeks and born in the holding, subsection of all animals to routine tuberculin test in annual intervals in accordance with Chapter Four.

(2) The Ministry may make the following amendments in the routine test frequency in whole or part of the country.

a) If on the 31<sup>st</sup> of December of each year, the annual rate of the cattle herds determined to be contaminated by the Bovine Tuberculosis is not more than 1 per cent among all herds in the designated region during the inspection period of the last two years, the frequency of the routine tests on herds may be extended to the intervals of two years and, on condition that they come from tuberculosis-free herds, male fatlings isolated in an epidemiological unit may be exempted from the tuberculin test and the authorized unit ensures that the male fatlings are not used for breeding and sent directly to slaughter.

b) If on the 31<sup>st</sup> of December of each year, the annual rate of the cattle herds determined to be contaminated by the Bovine Tuberculosis is not more than 0.2 per cent

among all herds in the designated region during the inspection periods of the last two two-years, the frequency of the routine tests on herds may be extended to the intervals of three years and/or the age of the animals subject to these tests may be increased to twenty four months.

c) If on the 31<sup>st</sup> of December of each year, the annual rate of the cattle herds determined to be contaminated by the Bovine Tuberculosis is not more than 0.1 per cent among all herds in the designated region during the inspection periods of the last two three-years, the frequency of the routine tests on herds may be extended to the intervals of four years or, on condition that the following requirements are satisfied, the authorized unit may not consider it necessary to subject the herds to tuberculin tests.

(3) All bovine animals should be subject to intra-dermal tuberculin test before joining the herd and the result should be negative.

(4) All animals slaughtered shall be examined with respect to lesions of tuberculosis; in the event that there such lesions, histopathological and bacteriological examinations shall be carried out with respect to the symptoms of tuberculosis. In the event of an increase in the disease level in the country or in a region, the Ministry may also increase the frequency of the tuberculin test application.

#### **Suspension, cancellation, monitoring and control of the tuberculosis-free status**

**ARTICLE 14** – (1) In case the requirements that are laid down in Article 13 cannot be satisfied, the tuberculosis-free status shall be suspended.

(2) In the event that it is recognized that one or more animals in the herd have positive results in the tuberculin test or there is suspicion of tuberculosis in post mortem examination, the animal considered to be the positive reactor shall be removed from the herd and slaughtered. Post mortem, laboratory and epidemiological examinations shall be carried out on the carcass of the positive reactor or the suspicious animal. The suspension status shall be maintained until the completion of all laboratory examinations. If, at the end of the laboratory examinations, the existence of tuberculosis is not verified, all of the animals older than six weeks in the herd shall be tested at least forty two days later the removal of the relevant animal from the herd and in case of a negative result, the official suspension of the tuberculosis-free status may be cancelled by the authorized unit.

(3) If there are animals in the herd that are suspicious for Bovine Tuberculosis, the status of suspension shall be maintained until the situation of the suspicious animals is clarified. Until their situation is clarified with another test to be carried out forty two days later or a post mortem examination and the laboratory examination, these animals shall be kept in a place separate from the other animals.

(4) In exception to the conditions laid down in the third paragraph, if the authorized unit carries out a routine herd test using the comparative tuberculin test specified in this Regulation and if no reactor animal is found out in the herd for the last minimum three years, the authorized unit may, on condition that no animals is allowed to be placed on the market until the clarification of the situation of the suspicious animals through another test to be carried out forty two days later, decide that the movements of the other animals in the herd are not restricted. In the event that a positive or suspicious reaction is observed in this last test, the conditions laid down in the second paragraph shall be

applicable. If the existence of the disease is determined afterwards, all animals sent from the holding shall be monitored until the last final herd test and shall be tested.

(5) In the event of existence of tuberculosis is determined through isolation of *Mycobacterium bovis* in the laboratory examination, the official tuberculosis-free status of the herd shall be cancelled.

(6) In case of the following conditions, the authorized unit shall cancel the bovine tuberculosis-free status of the herd;

- a) The requirements laid down in Article 13 cannot be satisfied any more,
- b) Observation of classical lesions of tuberculosis in post mortem examination,
- c) Strengthening of the possibility of infection in an epidemiological survey,
- ç) Observation of other factors that require control of bovine tuberculosis.

(7) Monitoring and control activities shall be carried out on herds that are deemed epidemiologically relevant by the authorized unit. All spaces and equipment shall be cleaned and disinfected and the withdrawal of the official tuberculosis-free status of a herd shall be maintained until all animals older than two weeks give negative results in the last two official tuberculin tests. The first of the tests shall be carried out in minimum sixty days and the second shall be carried out in minimum four months and maximum twelve months following the removal of the positive reactor from the herd. There should be at least sixty days between the first and the second tests.

#### **Announcement of the tuberculosis-free status**

**ARTICLE 15** – (1) In the event that the following requirements are satisfied, whole or part of the country shall be officially tuberculosis-free by the Ministry;

a) During a period of successive six years, the annual rate of the cattle herds that are confirmed to be infected with tuberculosis among all herds does not exceed 0.1 per cent and during a period of successive six years, on the 31<sup>st</sup> of December of each year, at least 99.9 per cent of the herds are tuberculosis-free.

b) Identification of each bovine animal in accordance with the Regulation 24829 of 28/07/2002 on the Description, Registration and Monitoring of Bovine Animals.

c) Submission of each slaughtered bovine animal to a post mortem examination.

ç) Continuation of the practices of suspension or cancellation of the official tuberculosis-free status.

(2) As long as whole or part of the country fulfils the requirements laid down in the first paragraph, it maintains its official tuberculosis-free status. However, in case of any symptom that might point out to any significant change in the situation of the whole or part of the country that is recognized to be officially tuberculosis-free, the Ministry may take a decision and may suspend or cancel the status until the fulfillment of the requirements laid down in such decision.

## CHAPTER FOUR

### **Identification of the Agent, Derma Tuberculin Test, Tuberculin Standards, Application of the Test, Official Tuberculin Tests, Supplementary Tests and Official Institutes and National Reference Laboratories**

#### **Identification of the agent**

**ARTICLE 16** – (1) *Mycobacterium bovis*, the agent of the bovine tuberculosis, can be determined through techniques of smear dyeing or immunoperoxidase and shall be confirmed by cultivation of the organism in primary isolation environment.

(2) The pathological material necessary for the culture and diagnosis of *Mycobacterium bovis* shall be taken from abnormal lymphoid nodes and parenchymatous organs such as lungs, liver, spleen, etc. In the event that no pathological lesion is determined in the animal, sampling shall be made from retropharyngeal, bronchial, mediastinal, supra mammillar, mandibular and some mesenteric lymphoid nodes.

(3) Identification of the isolates are generally made through specification of cultural and biochemical characteristics. Polymerase chain reaction (PCR) can also be used in determination of the *Mycobacterium tuberculosis* complex. DNA analysis techniques are faster and more reliable than biochemical methods in distinguishing the *Mycobacterium bovis* from other members of the *Mycobacterium tuberculosis* complex. The strains of *Mycobacterium bovis* can be distinguished by the genetic footprint technique and thus the origin and the models of contamination and transmission of *Mycobacterium bovis* can be identified.

(4) The techniques and environments used, their standardization and the assessment of the results shall be in accordance with the Guideline on OIE Standards for Diagnostic Tests and Vaccines (bovine tuberculosis).

#### **Derma tuberculin test**

**ARTICLE 17** – (1) The PPD (purified protein derivatives) Tuberculin having the characteristics defined in Article 18 shall be used as the official tuberculin in application of dermal tests following the execution of the procedure defined in Article 19.

#### **Tuberculin standards for cattle and poultry (bovine and avian)**

**ARTICLE 18** – (1) Tuberculin purified protein derivatives (PPD tuberculin, bovine or avian) is a preparation which is produced by a heat treatment of the reproductive and lysis products of *Mycobacterium bovis* or *Mycobacterium avium* and which is capable of generating a delayed type of hypersensitivity in an animal rendered sensitive against microorganisms of the same type.

#### **Production**

**ARTICLE 19** – (1) The preparation is produced from water soluble fractions obtained by heat treatment of *Mycobacterium bovis* or *Mycobacterium avium* (as appropriate) cultures, produced in a liquid synthetic environment, in free flow steam and then filtration of these cultures. It is isolated, washed and resolved by precipitation of active filtrate fraction, which is to a large extent composed of proteins. Antimicrobial

preservatives such as phenol, which do not result in false positive reactions, may be added. The final sterile preparation free from micro bacteria shall be aseptically distributed into sterile non-pressure glass vials that are later closed so as to prevent contamination.

### **Identification of the product**

**ARTICLE 20** – (1) The product is injected, via intra dermal way, at graded dose intervals, at different points of albino subjects, each of which is no less than 250 grams and which are appropriately sensitized. Twenty four – twenty eight hours later, reactions in the form of necrotizing or non necrotizing erythematous tubercles are observed at the injection point. The size and level of the reactions vary according to the dose applied. In non sensitized subjects, no reaction is observed in similar injections.

### **Tests**

**ARTICLE 21** – (1) The specifications of the tests are given below;

a) Ph: pH 6.5–7.5.

b) Phenol: If the preparation to be examined includes phenol, its concentration shall not be more than 5 gram/ liter.

c) Sensitization effect: Among subjects that have not been subject to any operation with any material that might have an effect on the test, a group of three subjects shall be used. Three intra dermal injections, each with a dose of an equivalent of 500 IU in 0.1 ml of the preparation, shall be made to each subject with an interval of five days. Fifteen – twenty one days following the third injection, these animals shall be injected with the same dose (500 IU) via intra dermal way and the same application shall be made to the control group of three subjects that have not been subject to tuberculin application beforehand. Twenty four – twenty eight hours following the last injections, no significant difference shall be observed in reactions in the two groups.

ç) Toxicity: Two subjects, each of which is not less than 250 grams and which have not been subject to any operation with any material that might have an effect on the test, shall be used. Each subject shall be injected, via subcutaneous way, with 0.5 ml of preparation to be examined. The animals shall be observed for seven days. During the observation period, no abnormal effect shall be observed in the animals.

d) Sterility: In compliance with the sterility test described in the monograph on veterinary vaccines in European Pharmacopeia, fourth edition, 2002.

### **Potency**

**ARTICLE 22** – (1) The potency of the purified protein derivative (bovine and avian) shall be determined by intra dermal injection into sensitized subjects of a serial dilution of a preparation produced in known concentrations of the reference preparation of tuberculin purified protein derivative (bovine or avian) calibrated in International Units and the preparation to be examined.

(2) For potency test, at least nine subjects, each of which is 400 gram – 600 gram, shall be used. For Mycobacterium bovis tuberculin, 0.0001 mg of wet mass of live Mycobacterium bovis AN5 stain, suspended in 0.5 ml of sodium chlorite R of 9

gram/liter, and, for avian tuberculin, an appropriate dose of inactive or live *Mycobacterium avium* shall be injected via deep intramuscular way. Minimum four weeks following the sensitization of the subjects, side flanks of the subjects shall be shaved and a sufficient area shall be opened for maximum four injections on both sides. Using isotonic phosphate buffer saline (pH 6.5-7.5) containing 0.005 gram/liter of polysorbate 80 R, dilutions of preparation to be examined and the reference preparation shall be prepared. At least three doses of the reference preparation and at least three doses of the preparation to be examined shall be selected. Doses which create a lesion of minimum 8 mm and maximum 25 mm shall be selected. The dilutions shall be randomly allocated to the injection areas in Latin square combination. Each dose shall be injected in fixed volumes of 0.1 ml or 0.2 ml via intra dermal way. Twenty four – twenty eight hours later, the diameters of the lesions shall be measured and the test result shall be calculated using statistical methods and assuming that the diameters of the lesions are in direct proportion with the logarithm of the tuberculin concentration.

(3) The test result shall not be considered valid if the reliability limits of the error ( $p=0.95$ ) is not less than 50 per cent and is not more than 200 per cent of the potency calculated.

(4) The potency calculated shall not be less than 66 per cent and more than 150 per cent of the potency specified for the bovine tuberculin.

(5) The potency calculated shall not be less than 75 per cent and more than 133 per cent of the potency specified for the avian tuberculin.

(6) The potency calculated shall not be less than 20 000 IU/ml for both (bovine and avian) tuberculins.

### **Storage**

**ARTICLE 23** – (1) Shall be kept away from light and at  $5\pm 3^{\circ}\text{C}$ .

### **Labeling**

**ARTICLE 24** – (1) The label shall contain the following;

- a) Potency per milliliter in International Unit,
- b) Name and amount of the substance added.

### **Administration of the test**

**ARTICLE 25** – (1) The tests specified below shall be official intra dermal tuberculin tests.

- a) Single intra dermal test: Bovine tuberculin shall be administered in single dose.
- b) Intra dermal comparative test: A bovine tuberculin injection and an avian tuberculin injection shall be administered at the same time.

### **Test dose**

**ARTICLE 26** – (1) One dose of the tuberculin to be injected shall not be;

- a) less than 2000 IU for bovine tuberculin,
  - b) less than 2000 IU for avian tuberculin.
- (2) The dose of each injection shall not exceed 0.2 ml.

### **Place of administration of tuberculin test**

**ARTICLE 27** – (1) The tuberculin shall be injected into the neck skin. In single intra dermal test, the test shall be administered to the middle part of the middle one third of the neck. In the event that an intra dermal comparative test is administered to the animal, the place of injection shall be in the middle one third of the neck; the avian tuberculin shall be administered into the above part and the bovine tuberculin shall be administered into the below part and the distance between these two points shall be 12.5 cm. In young animals whose neck does not have sufficient space on one side of the neck, one injection shall be made into the center of the middle one third of one side of the neck, and the other shall be made into the center of the middle one third of the other side of the neck.

### **Technique of the tuberculin test**

**ARTICLE 28** – (1) The hair on the area of injection shall be trimmed and the region shall be cleaned. Each area trimmed shall be measured by a compass holding a skin fold between the thumb and index finger of a hand and shall be recorded. Using a method for intra dermal administration of the tuberculin, an appropriate dose of tuberculin shall be injected. A short sterile needle with outside sloped borders, at the end of a graded syringe full of tuberculin, may be used to insert diagonally into the lower layers of the skin. Whether the injection has been made correctly shall be confirmed by finger sensing a small pea-like tubercle at the place of each injection. Seventy two hours ( $\pm$  four hours) following the injection, the thickness of each injection area shall be measured and recorded.

### **Assessment of reactions**

**ARTICLE 29** – (1) Assessment of the reactions, which shall be based on the increase in the thickness of the skin fold in the injection area observed in seventy two hours following the injection of tuberculin or tuberculins and the clinical observations, shall be made in accordance with the following;

a) Negative reaction: If there is a very limited tubercle, the thickness of the skin fold has not increased more than 2 mm, and if there has not occurred any diffuse or widespread edema, exudation, necrosis, pain or inflammation of lymphoid channels or lymphoid nodes, there is a negative reaction.

b) Suspicious reaction: If the clinical symptoms specified in paragraph (a) are absent and the increase in the thickness of the skin fold is more than 2 mm and less than 4 mm, then there is a suspicious reaction.

c) Positive reaction: If the clinical symptoms specified in paragraph (a) are observed or the increase in the thickness of the skin fold is 4 mm or more, then the reaction shall be deemed positive.

### **Single intra dermal test and its assessment**

**ARTICLE 30** – (1) A single intra dermal tuberculin test shall be assessed in accordance with the following;

a) Positive reaction: If, seventy two hours ( $\pm$  four hours) following the injection, the observed increase in the thickness of the skin fold at the place of administration is 4 mm

or more, and if a diffuse or widespread edema, exudation, necrosis, pain or inflammation of lymphoid channels or lymphoid nodes has occurred, there is a positive reaction.

b) Suspicious reaction: If, seventy two hours ( $\pm$  four hours) following the injection, the clinical symptoms specified for positive reaction are absent and the increase in the thickness of the skin fold at the place of administration is more than 2 mm and less than 4 mm, then there is a suspicious reaction.

c) Negative reaction: If, seventy two hours ( $\pm$  four hours) following the injection, there is a very limited tubercle, the thickness of the skin fold has not increased more than 2 mm, and if there has not occurred any diffuse or widespread edema, exudation, necrosis, pain or inflammation of lymphoid channels or lymphoid nodes, there is a negative reaction.

(2) Animals which give suspicious reaction in a single intra dermal test shall be tested again at least forty two days following the first test. Animals that do not yield negative reaction in the second test shall be considered to have given positive reaction to the test.

(3) Animals which give positive reaction in a single intra dermal test may be subjected to a comparative intra dermal test if there is a suspicion of false positive reaction or intervention reaction.

#### **Intra dermal comparative test and its assessment**

**ARTICLE 31** – (1) An intra dermal comparative tuberculin test shall be assessed in accordance with the following;

a) Positive reaction: If, seventy two hours ( $\pm$  four hours) following the injection, clinical symptoms such as diffuse or widespread edema, exudation, necrosis, pain or inflammation of lymphoid channels or lymphoid nodes are observed, or if the observed increase in the thickness of the skin fold at the place of administration of the bovine tuberculin is 4 mm or more and if the thickness of the skin fold at the place of administration of the bovine tuberculin is at least 4 mm more than the thickness of the skin fold at the place of administration of the avian tuberculin, then the reaction shall be considered to be a positive reaction.

b) Suspicious reaction: If, seventy two hours ( $\pm$  four hours) following the injection, the clinical symptoms specified for positive reaction are absent or the increase in the thickness of the skin fold at the place of administration of the bovine tuberculin is more than 2 mm and if the thickness of the skin fold at the place of administration of the bovine tuberculin is 1-4 mm more than the thickness of the skin fold at the place of administration of the avian tuberculin, then the reaction shall be considered to be a suspicious reaction.

c) Negative reaction: If, seventy two hours ( $\pm$  four hours) following the injection, the clinical symptoms specified for positive reaction are absent or the thickness of the skin fold has not increased more than 2 mm or if a positive or suspicious bovine reaction which result in an increase in thickness equal or less than that of positive or suspicious avian reaction has been observed, then the reaction shall be considered to be a negative reaction.

(2) Animals which give suspicious reaction in an intra dermal comparative test shall be subjected to a second test at least forty two days following the first test. Animals which do not yield negative reaction in the second test shall be considered to have given positive reaction to the test.

### **Suspension of the tuberculosis-free status of a herd and prohibition of entry into the market**

**ARTICLE 32** – (1) In cases defined below, the official tuberculosis-free status of the herd shall be suspended and the no single animal from the herd shall be allowed to enter into the market until the recovery of the situation of the animals in the herd;

a) Existence of animals that are deemed suspicious in single intra dermal tuberculin test,

b) Existence of animals that are deemed positive in single intra dermal tuberculin test but that are pending for a retesting with intra dermal comparative test,

c) Existence of animals that are deemed suspicious in intra dermal comparative test.

(2) In cases where, according to the legislation, animals should be subjected to intra dermal test before their movement, movements of animals which exhibit an increase of less than 2 mm in the thickness of the skin fold and which exhibit no clinical symptoms shall be allowed.

(3) In order to ensure determination of the number of infected or sick animals in a herd or a region, the Ministry may change test assessment criteria in order to attain enhanced test sensitivity by deeming all suspicious reactions obtained in single and comparative tuberculin tests positive reactions.

### **Supplemental tests**

**ARTICLE 33** – (1) In order to ensure determination of the maximum number of infected or sick animals in a herd or a region, the Ministry may allow implementation of the gamma-interferon test described for bovine tuberculosis in the OIE Guideline on Standards of Diagnostic Tests and Vaccines.

### **Tasks and responsibilities of official institutes and national reference laboratories**

**ARTICLE 34** – (1) Official Veterinary Control and Research Institutes, determined by the Ministry, as well as the National Reference Laboratories shall be responsible for official tests of tuberculins and reagents in order to ensure that these tuberculin tests and reagents are in compliance with the standards mentioned above.

## **CHAPTER FIVE**

### **Miscellaneous and Final Provisions**

#### **Regulative authority**

**ARTICLE 35** – (1) The Ministry is authorized to issue regulative procedures in order to ensure the application of this Regulation.

**Regulation repealed**

**ARTICLE 36** – (1) Bovine Tuberculosis Regulation, published in Official Journal No 16458 of 09/11/1978 has been repealed.

**Enforcement**

**ARTICLE 37** – (1) This Regulation shall enter into force on the date of its publication.

**Execution**

**ARTICLE 38** – (1) The provisions of this Regulation shall be executed by the Minister of Agriculture and Rural Affairs.