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**Regulation**

Ministry of Agriculture and Rural Affairs;

Regulation on Struggle with Brucellosis

**Chapter 1**

Objective, Scope, Legal Basis and Definitions

Objective

Article 1- (1) The objective of this regulation is to determine the applicable rules for gaining officially status of "herd which is free from brucellosis" with the subjects related to control of brucellosis and eradication.

Scope

Article 2- (1) This regulation covers the subject about protection of animal species like cattle, sheep and goats from brucellosis and rules and provisions of national eradication program.

Legal Basis

Article 3 - (1) This regulation has been prepared

- a) on basis of dated 8/5/1986 and decree law no 3285 on Animal Health and Surveillance Law
- b) in parallel with the 64/432/EEC numbered European Union Council Directive on "Animal Health Problems in cattle and pigs which affect the intra-community trade" and the 78/52/EEC numbered European Union Council Directive on "In cattle brucellosis, Tuberculosis and Accelerated Enzotik Leukozun Eradication for National Plan to Determining Community Criteria", the 91/68/EEC numbered "Animal Health Problems of sheep and goats which affect the intra-community trade" and "Placing on the Community's Financial Measures for sheep and goat brucellosis eradication" European Union Council Directive.

Definitions

Article 4 – (1) The terms used in this Regulation refer to

- a) Minister: The Minister of Agriculture and Rural Affairs,
- b) Ministry: Ministry of Agriculture and Rural Affairs,
- c) Fattening herd: herd consisting of bovine animals which are aimed to taken to the slaughterhouse
- ç) Region: a part of the country that has an area of at least 2000 km<sup>2</sup> and subject to control of authorized unit and contains at least one province
- d) Type B1 herds of cattle: Herds that are unknown of previous clinical and vaccination history and serological status
- e) Type B2 herd of cattle: Herds that are known of previous clinical and vaccination history and serological status and intended to be the status of type B3 and type B4 flocks in accordance with Appendix-3 and for this purpose being done routine monitoring tests
- f) Type B3 herd of cattle: Herds fulfilling the conditions in Attachment 1 and free from brucellosis (included animals vaccinated with Brucella S-19),

- g) Type B4 Herd of cattle: Herds fulfilling the conditions in Attachment 1 and officially free from brucellosis ( consisted of animals unvaccinated with Brucella S-19),
- ğ) Animals for stud or production: Bovine animals, with the exception of animals in Cultural and sporting activities, for purposes other than slaughter, grown for melioration, dairy or breeding,
- h) Thermal treatment: The process that covers heating other than boiling and after all type of applying alkaline phosphatase test immediately produces negative reaction,
- ı) Enterprise: An agricultural organization or facilities which owned as determined by current national rules and where animals are housed or are kept on a regular basis,
- i) Type M1 flock of sheep and goat: Herd that is unknown of previous clinical and vaccination history and serological status
- j) Type M2 flock of sheep and goat: Herds that are known of previous clinical and vaccination history and serological status and being aim to bring the enterprise the status of type M3 or M4 type in accordance with Appendix-4 and for this purpose being done routine monitoring test
- k) Type M3 Flock of sheep and goats: flock of sheep and goats fulfilling the conditions stated in Appendix-2 and free from brucellosis (included animals vaccinated with Brucella Rev-1),
- l) Type M4 Flock of sheep and goats: the flock of sheep and goats fulfilling the conditions in Appendix -2 and officially free from brucellosis (consisted of animals unvaccinated with Brucella Rev-1),
- m) Transport vehicles: Motor vehicles, parts of rail vehicles and aircraft set aside for loading, ships warehouses and containers use in land, sea and air transportation
- n) Official brucellosis test: Serological tests defined in Appendix-3 for cattle and Appendix-4 for sheep and goats,
- o) Officially brucellosis-free zone: A region of the country that fulfills the conditions contained in Appendix 1,
- ö) Official Veterinarian: Veterinarian assigned in Ministry of Agriculture and Rural Affairs central and provincial Administrative Organization,
- p) Herd: On an enterprise, an animal or group of animals kept as an epidemiological unit; on an enterprise, the presence of more than one group of animals each herd will comprise separate unit and will have the same health status,
- r) The Suspect Animal: Cattle, sheep and caprine animals that show indicating symptoms of Brucellosis disease but not having been done official confirmation,
- s) The authorized veterinarian: a veterinarian delegated by the Ministry of Agriculture and Rural Affairs and working under the authority and responsibility of an official veterinarian
- ş) Authorized unit: the provincial or district director of the Ministry.

## **Chapter 2**

### General Provisions

#### Precautions

Article 5 - (1) The presence or suspected case of brucellosis in cattle, sheep and caprine animals must be reported immediately to the unit.

(2) Procedures for the treatment of brucellosis in cattle, sheep and caprine animals are prohibited.

(3) A national reference laboratory for brucellosis is determined by Ministry. In laboratory personnel and laboratory, the necessary conditions must be fulfilled for implementation of diagnostic procedures. The duties and responsibilities of the national reference laboratories are indicated in Appendix-3.

## **Chapter 3**

## Special Provisions for Bovine Animals

### Eradication program for Brucellosis the Bovine Animals

Article 6 – (1) The program for eradication of brucellosis disease in cattle is prepared by the Ministry. The following measures shall be taken to the success of this eradication program;

- a) Eradication and prevention measures included in rate of the national cattle population are raised each year until cover the entire population.
- b) Compensation for animals, which are slaughtered because of bovine brucellosis disease after the official veterinary surgeon complete the necessary procedure, are paid to breeder as soon as possible.

### Registration and identification of bovine animals

Article 7- (1) In process of registration and identification of bovine animals

- a) The authorized unit provides a permanent registration and identification of cattle for the purpose of monitoring animal movements officially.
- b) The Authorized Unit creates the official classified record according to the health status of herds of cattle under the eradication program and keeps up date.

### Vaccination

Article 8- (1) In case of the implementation of vaccination, vaccination against brucellosis are provided under supervision official however the application of vaccination is terminated at the appointed time by the Ministry with the aim of transition from brucellosis to the status of the herds of free of brucellosis.

### Brucellosis in bovine animals

Article 9- (1) In case of a herd of animals suspected having brucellosis, authorized unit initiates official investigation in order to determine the presence of the disease as soon as possible. During the period the competent authorities perform the following points until results of investigations are received:

- a) Herd of cattle are kept under official supervision
  - b) This herd shall be prohibited the entry and exist of animals. Just with the permission of the competent unit and without delaying the movement of animals which will be delivered to slaughter is allowed. After isolated the suspected animals on the condition of obtaining a herd of fattening to shipped castrated animals to cutting the competent unit can allow possession at the farm.
  - c) The suspected animals in the herd are isolated
- (2) Matters stated in the first paragraph cannot be removed until the officially explanation of getting up out the the existence of brucellosis or the state of doubt in the herd or in the enterprise.
- (3) When Brucellosis has been detected in a herd officially, authorized unit shall take the necessary measures to prevent the spread of disease and especially provides the following aspects;
- a) The official veterinarian makes the necessary examination on the location of the disease and regulates the output report of the disease based on the laboratory report. Animal Health Security Committee takes the output decision of the disease and declares.
  - b) Apart from allowing by authorized unit to shipped cutting, entry and exit of such herd of bovine animals shall be prohibited. For movement of castrated cattle on the farm, the authorized unit can allow the isolation of infected animals in the herd and the cattle

which are considered to be infected by authorized unit and after marking pre-cutting, taking animals which has been castrated among fattening herds of animals before delivery to the slaughterhouse.

- c) Animals which is identified as officially brucellosis disease is caught and the animals which likely have been infected in the herd are isolated.
- ç) Animals officially identified as brucellosis disease is caught up are isolated and marked until slaughter.
- d) Milk of infected cows can be used for feeding the same farm animals after passing through suitable heat treatment processes
- e) Except for the purpose of being passed the appropriate heat treatment, milk obtained from infected cows in a herd cannot be transferred to milk processing plants and cannot be sold as raw milk.
- f) Except for detention for examination, fetus, newborn calves that died of brucellosis after birth or placenta shall be destroyed immediately.
- g) The straw, litter or other substances that have been in contact with the infected cow and calf, or the placenta are burned or buried after being disinfected and shall be destroyed immediately.
- ğ) Manure from the stables or the other compartments used by animals are kept strictly out of reach of farm animals. It shall be treated in a suitable disinfectant and kept for a period of at least three weeks. Disinfectant cannot be available for use in case of covering this manure with non-infected fertilizer or the soil. If cannot be collected at the same time with the manure, the liquid waste in compartments used by the infected animal is disinfected
- h) For removal of quarantine on the infected herd, after leaving infected animals from the herd at least thirty days later the first test and thirty to sixty days after the first test second test is done. When in the last two test of the entire herd results negative the third test is conducted six months later after the second test. The entire flock is removed from quarantine when this last test result in negative. By the time positive animal is detected on any one test, the test period is restarted.
- 1) Cattle with brucellosis found serologically positive are shipped to the cutting after being compensated. The regard of the meats of cutting cattle is applied according to relevant legislation. Carcasses, half carcasses, arms or thighs, parts and offal of infected cattle that are intended to be used as animal feed are treated as not lead to contamination. of carcasses will be destroyed and the facilities where they will be destroyed are officially controlled and measures shall be taken to prevent the spread of brucellosis.

#### Detection of brucellosis in bovine animals

Article 10 - (1) After bacteriological, allergic or serological examination, authorized unit gives officially test results and related liabilities that are about the official statement to the owner of bovine animals which is identified as carrying official brucellosis. Authorized unit make enable to being slaughtered the infected cattle under official supervision and on the condition of not to exceeding thirty days.

#### Brucellosis-infected areas

Article 11- (1) The Authorized Unit provides the fulfillment of the following issues on infected areas;

- a) After slaughtering infected animals which have been detected as official and before the supply of new animals, the barns, other compartments and the containers used for all animals, equipment and other materials shall be cleaned and disinfected veterinarian under control of an official veterinarian or the supervision of official veterinarian according to the instructions given by an authorized. Grasslands, which on this kind of

animals are found, are not used for a period of sixty days after the removal of these animals. Authorized unit can remove this prohibition exceptionally for animals that only have been castrated and will leave these pastures only due to the cutting and for animals that are intended to join the herds of fattening animals then being cut.

- b) After transportation of a flock of infected animals and animal materials or materials and substances which have been in contact with animals, all transport vehicles, containers and equipment are cleaned and disinfected. Loading areas used for this type of animals are cleaned and disinfected after used.
- c) Disinfectant and its concentration to be used are determined by authorized unit.

After cutting the animals infected with bovine brucellosis

Article 12- (1) Authorized unit provides the following points after the cutting of infected bovine animals;

- a) Entry and exist of any animals to in or out of infected herd are not allowed apart from animals that the competent unit allows only without delay the transfer for slaughter. The competent unit can allow the movement in the farm on the condition of joining castrated animals among herds of fattening animals and then aiming slaughtering.
- b) In order to find out the disease is eliminated in the herd, official brucellosis tests must be performed.
- c) Animals older than twelve months of age for breeding are not allowed to enter in infected bovine herds without being subjected to one or two official serologic tests in terms of brucellosis
- d) In the event that all the cattle in the herd were vaccinated with a vaccination identified by the Ministry at the young age of seven months, these tests need to be administered only in animals that have passed the age of eighteen months.

Classification of bovine animals in terms of brucellosis

Article 13 - (1) Authorized unit provides classification of herds of cattle in the form of B1 type, B2 type, B3 type and B4 type according to the health status of herds of bovine within the control and eradication program.

(2) In type B1 and type B2 herds, authorized unit ensures official serological tests until these herds gain the status of Type B3 and B4 herds.

Bovine animal transplants

Article 14 - (1) In terms of transplants B1 Type herds to B2 Type herds, authorized unit enables;

- a) to be subject animals older than twelve months to official brucellosis test within thirty days prior to the movement and to be kept ready the document, which is issued by an official veterinarian and indicates this action has been completed to possession during the transaction.
- b) To be isolated animals on coming to new herd and to be maintained them in isolation for a period of at least sixty days, to be subject to official brucellosis test before the adoption of Type B2 herd in the event animals are older than twelve months.

(2) In terms of transplants B2 Type herds to other B2 Type herds;

- a) If animals older than twelve months are being subject to official brucellosis test within thirty days prior to the movement and the document, which indicates this action has been completed, is issued by an official veterinarian, the authorized unit ensures the animals are not in contact with a lower health status animals during transport.
- b) In transporting animals between Type B3 and B4 flocks, the authorized unit provides compliance with the conditions on Appendix-1

#### Control and monitoring of movements of bovine animals

Article 15 – (1) Authorized unit ensures the operation of the following issues about control and monitoring of animal movements;

- a) The official control measures are taken to prevent re-infection of brucellosis in bovine herds that are eliminated from other sources.
- b) All flocks included in eradication program and movements of animal in these flocks are subject to official monitoring.
- c) Motion-control measures can be applied to free of brucellosis herds and officially Brucellosis-free herd within the framework of measures on the movement of animals out of these flocks.

### Chapter 4

#### Special Provisions on ovine and caprine animals

##### Eradication of brucellosis disease on ovine and caprine animals

Article 16 - (1) Eradication program shall be prepared for sheep and goat brucellosis. The following measures shall be taken to the success of this eradication program;

- a) The competent authorities that will implement the program and will coordinate should be determined.
- b) Sheep and goat breeding farms should be provided to be taken in recording.
- c) Identification and registration system that makes possible monitoring of sheep and goat movements must be established.
- ç) Sheep and goat enterprises will be classified as M1 type, M2 type, M3 type and M4 Type flocks of sheep and goats.
- d) In type M1 and M2 type flocks, official serologic tests should be done until these flocks have won status of M3 and M4 Type herd.
- e) Regions brucellosis vaccination will be done and banned should be determined.
- f) National budget reserved sheep and goat brucellosis eradication and the distribution the on the basis of items should be demonstrated.
- g) In the event of the suspected presence of brucellosis in an animal, the competent authority should ensure the necessary investigation made to detect the presence of disease as soon as possible. Until this investigation results, by the competent authority;
  - 1) Enterprise is kept under official surveillance.
  - 2) Except the animals of which the authorized unit allows cutting transfer, all animal movements to entity and out of the operation are prohibited.
  - 3) The suspect animals within the enterprise are isolated.
- ğ) Until disappearance status of suspected presence of brucellosis in the concerned operation has been identified as an official, the matters mentioned in subparagraph (g) continue.
- h) As the presence of brucellosis in a herd officially is detected, the competent authority takes the following measures to prevent the spread of the disease;

1) When sheep and goat brucellosis disease is detected in accordance the laboratory report, the official veterinarian regulates the output report of the disease. Animal Health Security Committee takes and declares the disease the output decision.

2) All movements except the animals that authorized unit permits the transfer of cutting are prohibited to in or out of these herds.

3) The animals in which the presence of brucellosis has been identified as the official are isolated and marked until slaughter time.

4) Other animals in the herd shall be subject to official brucellosis test. The quarantine measures taken because of the disease brucellosis in sheep and goats is removed after the disinfection done in thirty days later the last throwing baby.

5) Milk from infected animals in a herd is isolated and it can be evaluated only in this enterprise for use feed material or cheese by being isolated in a suitable way and subjected to suitable heat treatment process.

6) Milk obtained from non-infected animals in an infected herd can be taken out of enterprise only after appropriate heat treatment process.

7) Except for the detention in order to inspection, stillborn baby, fetus or placentas due to brucellosis are destroyed.

8) Straw, litter or other substances that have been in contact with infected animals or animals or placentas are burned or destroyed immediately and buried after disinfected with a disinfectant approved by the competent authority. However, in the case of materials to be used, they shall be disinfected with a disinfectant that is mentioned before reused.

9) Manure from the stables or the other compartments used by animals are kept strictly out of reach of farm animals. It is disinfected through an appropriate disinfectant approved by the competent authority and kept for a period of at least three weeks. This fertilizer by no means is used to spread on a commercial garden plants. If this manure is covered with in a layer of soil, disinfectants may not be used. Unless it are gathered at the same time with the manure, the liquid waste compartments infected animals used is also disinfected.

(2) The authorized unit officially provides the official statement about the test results and related obligations to owner of animals detected of carrying brucellosis after bacteriological or serological examination. Infected animals are shipped to cutting under official supervision as soon as possible and within at the latest thirty days. the competent authority can extend this period to of forty-five days by taking into account of the various challenges. On meat of sheep and goat, procedure shall be applied according to the relevant legislation.

(3) The following considerations should be provided for the success of eradication programs;

a) After slaughtering infected animals which have been detected as official and before the supply of new animals, the barns, other compartments and the containers used for all animals, equipment and other materials shall be cleaned and disinfected veterinarian under control of an official veterinarian or the supervision of official veterinarian according to the instructions given by an authorized. Grasslands, which on this kind of animals are found, are not allowed to be reused for a period of sixty days after the removal of these animals.

b) After moving a flock of infected animals and substances of animals or materials which have been in contact with animals, all transport vehicles, containers and

equipment are cleaned and disinfected. Loading areas used for this type of animals are also cleaned and disinfected after used.

c) Disinfectant and its concentration to be used are determined by authorized unit.

ç) Entry and exist of any animals to in or out of the enterprise are not allowed except for allowed by the competent unit to deliver in order to the cutting.

d) Until it is determined that these infected herd has been eliminated from brucellosis official brucellosis tests are performed.

e) Unless animal that is older than six months and remains for breeding purposes after the slaughter of infected animals is tested one or more to an official brucellosis examination, a new breeding animals entry cannot be made to the enterprise. In the event that under seven months all the sheep and goats in the herd were vaccinated with a vaccination identified by the Ministry, these tests need to be applied only in animals that have passed the age of eighteen months.

f) By the time brucellosis has been diagnosed in sheep or goats, who returned from a plateau, if the infected animal is from grazing, breeding and contact with sheep and goats of other enterprise in the plateau in regular intervals, all animals of the flock and particularly all flocks that give animals to pasture and milking are considered as major infected flock and the official brucellosis tests are applied until it is determined that the disease is eliminated.

## Chapter 5

### Miscellaneous and Final Provisions

#### Regulation Authority

Article 17- (1) Ministry is authorized to issue the regulatory transactions to ensure the implementation of this Regulation.

#### Enforcement

Article 18- (1) This Regulation shall enter into force on the date of publication.

#### Execution

Article 19- (1) The Minister of Agriculture and Rural Affairs shall execute the provisions of this Regulation.

#### Appendix-1

### OFFICIALLY FREE OF BRUSELLOSIS AND BRUCELLOSIS-FREE HERDS OF CATTLE

#### A- Bovine animals

1- Mentioned "bovine animals" refers to except for male animals all bovine animals that from brucellosis-free flocks for the purpose of fattening and guaranteed by the competent authority to not being used in breeding and ultimately that will be sent directly to the slaughterhouse.

#### B- Officially bovine brucellosis-free status

1- A cattle herd is officially free from brucellosis in the presence of the following conditions;

a) Vaccinated bovine against brucellosis are not maintained in the herd. (the exception of female animals vaccinated prior to at least three years).

b) Clinical symptoms of brucellosis should not be seen in all cattle in the herd for at least six months.

c) In the herd, all cattle over the twelve month should be undergo to one of the following testing regimes in accordance with Appendix-3 and should give negative results;

- (1) Two official serological tests mentioned in Appendix-3 and which are done in more than three months and less than twelve months intervals should be negative.
  - (2) After the official serological test defined in in Appendix-3 at the earliest six weeks later, milk samples in the three tests which are applied three-month intervals should be negative.
- ç) In accordance with Appendix-3, in the serum agglutination test of over the twelve month cattle that is incoming from flock officially brucellosis-free and involved in the herd should give less than 30 IU 'titre in each ml or in accordance with the procedure established by the Ministry the test applied within thirty days prior to joining the herd or the period of thirty days from joining the herd should give a negative result. In the second case, animal / animals shall be physically isolated from other animals and until proved to be negative their contact with other animals, either directly or indirectly is prevented.

#### C - Continuation of officially brucellosis cattle disease-free status

- 1- A herd of cattle maintains the status of brucellosis-free in case of fulfillment of the following conditions;
- 2- Every year tests mentioned below and performed in accordance with Appendix-3 should give a negative result.
  - a) The milk ring test that is applied three times at least three-month intervals.
  - b) The milk ELISA tests that is applied three times at least three-month intervals.
  - c) Two milk ring tests made with an interval of at least three months and subsequently, at least six weeks later the official serological test indicated in Appendix- 3.
  - ç) Two milk ELISA test made with an interval of at least three months and subsequently, at least six weeks later the official serological test indicated in Appendix-3.
  - d) Two official serological test made with an interval of at least three months and at last twelve months.
- 3- In case of the absence of official status of a brucellosis-free throughout the country or a section of the country and however the inclusion of all cattle herds in the official program of struggling brucellosis the ministry can change the frequency of routine tests as in the following.
  - a) In the event of infection in less than 1% of cattle herds every year two milk ELISA test with an interval of at least three months or a serological test may be adequate.
  - b) In the event at least 99.8% of cattle herds is recognized as officially brucellosis-free for at least four years, the period between inspections may be extended to two years on condition that all animals over the age of twelve-month is tested or test procedure may be limited to animals over twenty-four month the as long as the test is continued to be performed every year. Controls must be made by using one of the official serological tests as indicated in Appendix 3.
- 4- over the twelve month cattle that is incoming from flock officially brucellosis-free and involved in the herd gives less than 30 IU brucellosis titre in each ml or the test applied within thirty days prior to joining the herd or the period of thirty days from joining and test approved by the Ministry will give a negative result. In the second case animal / animals will be physically isolated from other animals and until proved to be negative their contact with other animals either directly or indirectly will be prevented.

- 5- Without being bound by subparagraph 4 of title C, cattles in a brucellosis-free herd can be incorporated into officially brucellosis-free herds if they are at least eighteen months and vaccinated against brucellosis and providing that vaccination is made prior to further time than a year. It is essential that such animals has given brucella agglutination titers lower than 30 IU in each ml up to thirty days before joining the new herd and complement fixation test or another test approved by the Ministry has given a negative result. For all that when a female cattle will be included in a officially brucellosis-free herd from a brucellosis-free herd, in accordance with preceding conditions a herd will be accepted as brucellosis-free herd for two years following the date of including the last vaccinated animal into herd.

Ç- Officially suspension, cancellation of the brucellosis bovine disease-free status

- 1- Free status of brucellosis in a cattle herd shall be officially suspended in the following cases;
  - a) Failure to follow conditions stated in B and C titles of this annex
  - b) When suspecting one or a greater number animal of brucellosis on the basis of the results of laboratory tests or clinical basis and in the event of cutting suspected animal or isolation by preventing direct or indirect contact with other animals.
- (1) On the event of slaughtering suspected animal and the absence of animal in testing to be unsuspended in two agglutination test made in accordance with Appendix-3 all the animals over the age of twelve months in the herd need to give less than 30 IU brucella agglutination titer for each ml. First test is applied at least thirty days after the removal of infection animals and the second test is made at least sixty days later.
- (2) In the case of isolation suspected animals from other animals in the herds, for re-participation of animal in the herd and to regain the status it must give a agglutination titer lower than 30 IU for each ml in serum agglutination test and complement fixation test must give a negative result or the combination of another test approved by the Ministry for this purpose must give a negative result.
- 2- The official brucellosis-free status of the herd will be canceled providing that it is determined the presence of brucellosis infection in a herd on the basis of the results of laboratory testing and epidemiological investigations. In order to regain the status of the herd, all cattle in the herd during the epidemic will be subject to the herd control test. All cattle over the age of twelve months must give negative result in the first test made at least thirty days later after the removal of the infected animals from the herd and in the second test made sixty days later after the first test. The last control about pregnant female animals during the epidemic must be done at least twenty-one days later after the last animal pregnant during the epidemic calves.

D - Bovine brucellosis disease-free status

- 1- A herd of cattle is free from brucellosis by the time conditions mentioned in the subparagraph (b) and subparagraph (c) of the title B in this Appendix has fulfilled and vaccination is applied to as prescribed in the following;
  - a) The female cattle were immunized with the S-19 live vaccine (live strain) at the age of three - six months.
  - b) In the serum agglutination test cattle that is under thirty months of age and vaccinated with the live S-19 vaccine gives up to 30 IU and 80 IU down the agglutination titer in each ml. however in this case if they are vaccinated before less than twelve months in the complement fixation test these animals must give lower results than 30 EEC units or in all other cases they must give results lower than 20 EEC units.

E - Continuation of bovine brucellosis disease-free status

- 1- A cattle herd maintains officially brucellosis-free status in the event of fulfillment the conditions mentioned below;
  - a) It must be subject to one of testing regimes stated the second paragraph of the title C of this Annex.
  - b) Cattle entering the herd must have the requirements specified in the fourth paragraph of title C of this Appendix or;
- (1) In the event of having arrived from brucellosis-free herd and up to thirty days before the arrival of the cattle over the twelve-month into herd or in case of isolation after entering the herd test it must give agglutination titer of lower than 30 IU for each ml in the serum agglutination and in the complement fixation test made in accordance with Appendix-3 it must give negative result or;
- (2) Coming from brucellosis-free herd, being under age of thirty months, vaccinated with live S-19 vaccine must give the agglutination test more than 30 IU but lower than 80 IU for each ml. in the serum agglutination test. However, in this case in the complement fixation test female animals vaccinated before the period less than twelve months must give low results from 30 EEC units and however in all other cases it need to give low results from 20 EEC units.

F- Suspension, cancellation of bovine brucellosis disease-free status,

- 1- Free status of brucellosis in a cattle herd shall be suspended in the following cases;
  - a) Failure to follow conditions stated in D and E titles of this annex
  - b) If one or greater number of cattle over thirty months is suspected to carry brucellosis on the basis of the results of laboratory testing and epidemiological investigations, suspect animals are slaughter or they are isolated by preventing direct or indirect contacts with other animals. In the event of isolation of animals, to participate in the herd again they must give agglutination titer lower than 30 IU each ml. in the serum agglutination test and in the complement fixation test or other tests approved by the Ministry they must be give a negative result. In the case of the absence for test and slaughter of animals, to remove the state of suspension in accordance with Annex-3 in the all cattle older than twelve months, agglutination titer lower than 30 IU must be obtained from each ml. in two serum agglutination test. The first of the tests will be performed thirty days later after the removal of animals and the second test shall be sixty days after this.
  - c) If animal which tests mentioned in subparagraph (a) and subparagraph (b) of this title will be done, is younger than thirty months and if they were immunized with S-19 live vaccine, in their serum agglutination test the results higher than 30 IU and lower than 80 IU will be accepted as negative. However, in this case, in the complement fixation test for female animal which is vaccinated in the period less than twelve months low result from 30 EEC units must be gotten or in all other cases low result from 20 EEC units must be gotten.
- 2- If it is determined the presence of brucellosis infection in a herd on the basis of the results of laboratory testing and epidemiological investigations, brucellosis-free status of this herd will be canceled. In this situation to gain back the status of the herd all cattle which is in the herd during the epidemic shall be subject to the herd control test. All unvaccinated cattle over the age of twelve months must give a negative result in the sixty-day interval two tests which of the first is done at least thirty days after removal of infected cattle(s) from the herd. The last control about pregnant female animals during the epidemic must be done at least twenty-one days later after the last animal pregnant during the epidemic calves. If the animals that the mentioned tests

shall be made in, is younger than thirty months of age and were not immunized with S-19 live vaccine, in their serum agglutination test the results of brucella titres higher than 30 IU and lower than 80 IU for each ml. will be accepted as negative. However, in this case, in the complement fixation test for female animal which is vaccinated in the period less than twelve months low result from 30 EEC units must be gotten or in all other cases low result from 20 EEC units must be gotten.

#### G - Officially bovine brucellosis-free zone

- 1- According to the rules established by the Ministry, country or a part of the country shall be announced as officially free of brucellosis in the case of fulfillment of the conditions mentioned below;
  - a) There must be no case of waste due to brucella infection for at least the last three years, *B. abortus* has not been isolated and as been calculated on thirty-one December of each calendar, year at least five years consecutively 99.8% of the herd must gain officially brucellosis-free status.
  - b) Each cattle will be kept in record through eartag.
  - c) Cases of waste must be notified compulsorily and the necessary examination will be made.
- 2- To be announced the country or a part of the country as an officially free of brucellosis, further;
  - a) It will continue to be provided the conditions stated in subparagraphs (a) and (b) of the first paragraph of this the title and, the notification of the waste cases suspected to be caused by brucellosis and investigation will be mandatory.
  - b) Every year in the first five-year period after the acquisition of statute the at least 20% of herds must give negative results for the serological tests made to all cattle over twenty-four month in accordance with Appendix-3 or for the examination of milk samples made in accordance with Appendix-3 negative results must be taken in the dairy cattle herds.
  - c) The cattle suspected to be infected with brucellosis is reported to the competent department and it is subject to the official epidemiological investigation of official in terms of brucellosis including microbiological examination from at least two official serological blood test and appropriate examples.
    - ç) In the suspicious period that will continue until getting a negative result in tests stated in subparagraph (c) of this title, officially brucellosis-free status of the herd shall be suspended. In addition, the official brucellosis-free status of other herds which are in contact with suspected cattle and linked epidemiologically also shall be suspended.
    - d) In the case of an epidemic of brucellosis spreading in the region declared free of brucellosis, all cattle shall be slaughtered. Appropriate tests are applied in the animals of the remaining suspected types and shelters and equipment are cleaned and disinfected.
- 3- In accordance with the objectives set out in Appendix- 1 a serological test means a serum agglutination test or buffered brucella antigen test, complement fixation test, plasma agglutination test, plasma ring test, micro-agglutination test or individual blood ELISA test as described in Appendix- 3. Other diagnostic tests which are approved in accordance with the procedure established by the Ministry and described in Appendix- 3 are also accepted for the purposes set out in Appendix-1. The milk test means a milk ring test in accordance with Appendix-3 or ELISA in the milk.

**OFFICIALLY FREE OF BRUCELLOSIS (B.melitensis) AND BRUCELLOSIS- FREE FLOCK OF SHEEP OR GOATS**

**A- Officially sheep and goat brucellosis disease-free status**

- 1- A sheep or a goat flock is officially free from brucellosis (B. melitensis) in the presence of the following conditions;
  - a) In all animals which is sensitive to brucellosis (B. melitensis), clinical symptoms of brucellosis (B. melitensis), is not observed at least last twelve months.
  - b) Except for animal that is vaccinated with young Rev-1 vaccine at least two years ago and with another approved vaccine in accordance with the procedure established by the Ministry vaccinated sheep and goats must not be maintained.
  - c) All sheep and goats over six months must give a negative result in the two tests in accordance with Appendix-4 and which are made in intervals of six months or more.
  - d) After the tests stated in the subparagraph(c) of this title, just the sheep and goats who was born in the herd must be maintained or consist sheep and goats coming from officially free of brucellosis flock.

**B - The continuation of official sheep goat brucellosis disease-free status**

- 1- A sheep and goat herd continues to maintain officially brucellosis-free status in the event of fulfillment the conditions mentioned below
- 2- in the Officially brucellosis (B. melitensis)-free sheep or goat enterprises which is not located in the region recognized as officially free of brucellosis and the enterprise that has entry of animals after determined their purity, sheep and goats in a representative number of animals over a six-month shall be subject to a control in a year. To carry on the official brucellosis (B. melitensis)-free status of the herd, the results of these tests must be negative. In each enterprise representative number of animals to be tested must be determined as follows.
  - a) Over six months noncastrate male animals
  - b) All animals that are shipped to enter after the last test.
  - c) 25% of the females which are reached reproductive age (sexual maturity); or for milking animals, a minimum of 50 animals for each enterprise; in case of having fewer than 50 female animals, all female animals.
- 3- For officially brucellosis-free region that has more than 99% of the sheep or goats enterprises declared as officially free from brucellosis (B. melitensis), control frequency of officially brucellosis-free sheep and goat enterprises can be extended three years, providing enterprise which is not officially free of brucellosis is brought under official control or included in the eradication program.

**C - Cancellation of officially sheep and goat brucellosis disease-free status**

- 1) The official brucellosis-free status of sheep and goat herd;
  - a) In the event of the suspicion that one or more sheep or goats carry brucellosis (B. melitensis), official brucellosis-free status of enterprise shall be revoked by the competent authority. However, if the animal or animals shall be slaughtered immediately or if it is isolated until the official determination of the disease or if the suspicion of the disease officially removed, status may be suspended conditionally.

- b) For by the competent authority the removal of state of conditionally suspension that occurs when brucellosis (*B. melitensis*) is detected, all infected animals or animal species which are susceptible to infection must be slaughtered and all animals older six months in the enterprise must give a negative result in the two test made in accordance with Appendix-4 and intervals of at least three months or more.
- 2- If infected herd are located in a region defined as officially free of brucellosis (*B. melitensis*);
  - a) Authorized unit has all infected animals in the herd and all animals belonging to the animal species that is susceptible to infection slaughtered.
  - b) Authorized unit has epidemiological examination performed and makes the tests stated in the subparagraph (b) of the first paragraph of this title applied to the herd that has epidemiological connection with infected herds.
- 3- In the event that epidemic brucellosis is detected within the context of B title in this Appendix, by reviewing the conditions for new brucellosis (*B. melitensis*) epidemic ministry can decide to the suspension or cancellation of the region's status when considered necessary. In case of cancellation, conditions for the acquisition of status again will be determined in accordance with the same procedure.

Ç- Joining of the new animals to the officially brucellosis disease-free sheep and goat herd

- 1- New animals are not allowed to join in the officially brucellosis disease-free sheep and goat herd as long as these conditions are not fulfilled.
  - a) Sheep and goats from the officially brucellosis disease-free sheep or goat herd must be maintained.
  - b) They are required to come from brucellosis-free enterprise and;
    - (1) They must be defined by using eartag as determined by the Ministry.
    - (2) They must be never vaccinated against brucellosis or if they are vaccinated, it must have happened prior to a period of two years. If female animals over the age of two were immunized before seven months, they can join to the herd.
    - (3) They must be isolated under official surveillance in the herd and during this type of isolation they must give negative result in the two tests made in accordance with Appendix-4 with and an interval of at least six weeks.

D - Officially sheep and goat brucellosis-free zone

- 1- In order to be defined by the Ministry as officially brucellosis-free, a country or a part of country;
  - a) At least 99.8% of sheep and goat enterprises located in the region must be officially brucellosis-free enterprises or,
  - b) It is required to fulfill the following conditions.
    - (1) At least five year the sheep and goats brucellosis must be compulsorily notifiable disease.
    - (2) At least five year sheep and goat brucellosis must not be officially identified
    - (3) Vaccination must be prohibited for at least three years
  - c) The conditions determined by the Ministry must be followed in the country or a part of the country.
- 2- Besides the fulfillment of conditions mentioned above,
  - a) Within the first year after identification of the country or a part of the country as the officially free of brucellosis (*B. melitensis*), when there are random checks in the level of flock or slaughterhouses, enterprise with 99% confidence interval must be detected less than 0.2% of them infected or at least 10% of all sheep and goats

over six-month is required to give a negative result in the tests made in accordance with Appendix-4.

- b) Within the second year after identification of the country or a part of the country as the officially free of brucellosis (*B. melitensis*) when random checks are made in the level of enterprise or slaughterhouses, enterprise with 95% confidence interval must be detected less than 0.2% of them infected or at least 5% of all sheep and goats over six-month is required to give a negative result in the tests made in accordance with Appendix-4.
- c) In the conditions set out in subparagraphs (a) and (b) of this title, changes can be made in accordance with the procedure established by the Ministry.

#### E – Sheep and goat brucellosis (*B. melitensis*) disease-free status

- 1- A flock of sheep or goat is free from brucellosis (*B. melitensis*) in the presence of the following conditions;
  - a) All animals which is sensitive to brucellosis (*B. melitensis*) must not have clinical symptoms of brucellosis (*B. melitensis*) at least last twelve months.
  - b) All or a part of sheep and goats is vaccinated with young Rev-1 vaccine with another approved vaccine in accordance with the procedure established by the Ministry. Animals must be vaccinated at the age of three - six months.
  - c) All vaccinated sheep and goats over eighteen-month in the flock must give a negative result in two tests made with an interval of at least six months and in accordance with the Appendix-4.
  - ç) All sheep and goats which are unvaccinated and over six months during the test flock must give a negative result in two tests made with an interval of at least six months and in accordance with the Appendix-4.
  - d) After application of brucellosis test in the flock, all sheep and goats that join into the flock must be born in this enterprise or must come from brucellosis-free flock.
- 2- After determined as brucellosis-free by authorized unit, the conditions -mentioned above must continue to be fulfilled.

#### F- Pure statue proceeding from sheep, goat brucellosis disease (*B. Melitensis*)

1 – Sheep and goat herd may keep the pure statue from brucellosis when it fulfills the following conditions;

- a) Amount of animals which represent the current sheep and goat number in herd will be tested one in a year. To keep the statue of management depends on tests to be negative.
- b) Representative number of animals in the herd, which will be tested, should be set according to following conditions.
  - (1) All male animals which are unsteered and not vaccinated over six months.
  - (2) All male animals which are unsteered and not vaccinated over eighteen months.
  - (3) All animals which are brought to herd after the latest test.
  - (4) 25 % of female animals which are at the age of sexual maturity or breeding, or, minimum fifty animals from milking animals, or, if there are less than fifty female animals, all female animals will be tested.

#### G - Pure statue cancelation from sheep, goat brucellosis disease (*B. Melitensis*)

1- Pure statue from brucellosis of sheep, goat herd;

- a) If there is any suspicion that one or more sheep or goats have brucellosis disease (*B. Melitensis*), pure statue from brucellosis of sheep, goat herd will be cancelled by official body. However, when animal or animals are immediately destroyed or isolated until the

official recognition of disease or suspicion of disease is removed officially, statue can be suspended on conditioned.

b) In order to remove suspended condition by official body when brucellosis disease (B. Melitensis) is identified, all infected animals or infection sensitive animal species will be slaughtered and there must be a negative results from the two tests which are applied for minimum three or at more regular intervals according to following conditions based on Appendix-4.

(1) All animals over eighteen months which are not vaccinated

(2) All animals over six months which are not vaccinated.

Ğ – Adding new animals to pure herd from sheep, goat brucellosis disease (B. Melitensis)

1 – When the following conditions are not applied, adding new animals to pure herd from sheep, goat brucellosis disease (B. Melitensis) is not permitted.

a) Sheep and goat should be come from officially pure management or pure from brucellosis disease (B. Melitensis).

b) Other animals which are come from some other factories different from “article a” above stationed should perform the following conditions.

(1) Animals must be identified with ear-ring determined by Ministry.

(2) All animal species which are sensitive to brucellosis disease (B. Melitensis) should come from the herd in which there are no sing of brucellosis disease (B. Melitensis) minimum for the last twelve months.

c) Not being vaccinated within the last two years or,

(1) Being isolated under the supervision of official veterinary surgeon as long as they stay or within this period they must give at least two negative test result which are applied minimum distance of six months in accordance with Appendix 4 or,

(2) Two or three months animals should be vaccinated with Genç Rev-1 or any other vaccination of which procedure convenience identified by Ministry is approved, fifteen days before they come into arrival place or herd.

H – Gaining official pure herd statue brucellosis disease (B. Melitensis) of pure herd of sheep, goat from brucellosis disease (B. Melitensis)

1- Gaining official pure herd statue brucellosis disease (B. Melitensis) of pure herd of sheep, goat from brucellosis disease (B. Melitensis) is possible when minimum two years period is completed by applying following conditions;

a) There should be no animal which are vaccinated for brucellosis disease (B. Melitensis) minimum two years ago in the herd.

b) After the period of two years, at the test which are applied in accordance with appendix-4, all animals over six months should take negative results.

c) For two years period, accordance with conditions which are stated at title Ğ of appendix should be kept.

### Appendix 3

## IDENTIFYING THE FACTOR, BRUCELLOSIS TESTS AND REFERENCE LABORATORIES

### A – Identifying the factor

1- Showing brucellosis organism morphologies with immune specific paintings or modified acid-fast in milk, abort material or vaginal fluxion are the sign of existence of brucellosis especially when it is supported with serologic tests. After the isolation specie and biovar will be identified with the phage lysis and/or oxidative metabolism tests, cultural, biochemical and serologic criteria. Techniques and tools in used, their standardization,

interpreting of results will be in accordance with current practices which are stated at OIE Diagnostic Tests and Vaccinations Standards Guide for the diagnosis of cow brucellosis and sheep, goat brucellosis.

## B – Brucellosis Tests

### 1- Immunologic Tests

#### 2- Standards

a) Brucellosis abortus biovar 1 Weybridge strain Numbered 99 or USDA strain 1119-3 will be used while preparing Rose Bengal Test (RBT), serum agglutination test (SAT), complement fixation test (CFT) and milk ring test (MRT).

b) OIE international reference standard serum (OIEISS) is the standard reference serum for RBT, SAT, SFT and MRT. This serum is also named as WHO second international anti-brucellosis abortus serum (ISAbS).

c) Standard reference serums for ELISA tests are below mentioned;

(1) OIEISS,

(2) OIE ELISA weak positive standard serum (OIEELISAWPSS)

(3) OIE ELISA strong positive standard serum (OIEELISASPSS)

(4) OIE ELISA negative standard serum (OIEELISANSS)

ç) OIEISS, OIEELISAWPSS, OIEELISASPSS and OIEELISANSS are international primary standards which prepare secondary international reference standards which are obligatory for each test.

3- Enzyme-linked immunosorbent assay (ELISA) which is used to determine cow brucellosis in serum or milk and other binding tests are as follows;

a) Materials and indicators, technique in used and interpreting the results must be in consistent with the principles of OIE Standards Hand Book for Diagnostic Tests and Vaccinations or at least it should include laboratory and diagnostic performances.

b) Standardization of Test.

(1) Standardization of the test procedure for individual serum examples:

1/150 pre-dilution of OIEISS or 1/2 pre-dilution of OIEELISAWPSS or 1/16 pre-dilution of OIEELISASPSS which was prepared in negative serum (or in negative bulk serum samples) must give positive reaction.

1/600 pre-dilution of OIEISS or 1/8 pre-dilution of OIEELISAWPSS or 1/64 pre-dilution of OIEELISASPSS which was prepared in negative serum (or in negative bulk serum samples) must give negative reaction. OIEELISANSS should always give negative reaction.

(2) Standardization of test procedure of pool serum samples:

1/150 pre-dilution of OIEISS or 1/2 pre-dilution of OIEELISAWPSS or 1/16 pre-dilution of OIEELISASPSS which was prepared in negative serum (or in negative bulk serum samples) and was rediluted with negative serum as much as number of samples making pool must give positive reaction. OIEELISANSS should always give negative reaction. Test should be sufficient to identify enough infection sign of one animal within the animal group which are made pool from their serums.

(3) Pool created milk and whey test procedure standardization:

1/1000 pre-dilution of OIEISS or 1/16 pre-dilution of OIEELISAWPSS or 1/125 pre-dilution of OIEELISASPSS which was prepared in negative serum (or in negative bulk serum samples) and was rediluted with negative milk as the proportion of 1/10 must give positive reaction. OIEELISANSS which was rediluted in negative milk should always give

negative reaction. Test should be sufficient to identify enough infection sign of one animal within the animal group which are made pool from the samples of milk or whey.

4- Conditions of usage of ELISA tests for cow brucellosis diagnostic;

a) At serum samples, ELISA tests must be equal or higher than RBT or CFT by using above mentioned calibration conditions, by considering epidemiological situation in diagnostic sensitivity of ELISA.

b) At the pool created milk samples, ELISA tests must be equal or higher than MRT by using above mentioned calibration conditions, by considering the average, expected and best system of epidemiological situation in diagnostic sensitivity of ELISA.

c) For the situations such as determining and protecting herd statue of ELISA tests, certification oriented, test results for creating a pool from serum samples must be created to show that they are related to several animals in the pool without giving a room for any doubt. At the individual serum samples which are taken from animals confirming tests are obligatory to apply.

ç) ELISA tests can be used for milk sample which is prepared from milk of 30% of milk cows on milking in a herd. When this method is used, health oriented all precautions must be taken to correlate without giving any room to suspicion for related animals which are milked, or sample taken. Confirming tests for serum samples of isolated animals are obligatory to apply.

5- Complement Fixation Test (CFT)

a) It is consisted of bacterial suspension inside of antigen, phenol-saline (NaCl %0,85 (m/v)) and % 0,5 phenol in veronal buffer (v/v) (volume/volume). Delusion factor which is used for distributing of antigens in concentrate situation should be pointed at the bottle label. Antigen must be kept at 4C degree and not to be frozen.

b) Inactivation of cow serum must be made between 56C and 60C degrees and for a thirty to fifty minutes period.

c) In order to create real reaction in the test procedure, it needs to be use higher dose of complement dose than minimum dose which is needed for total hemolysis.

ç) Following controls must be made for each time while applying complement fixation test.

(1) Anti complementary effect of serum

(2) Control of Antigen

(3) Control of sensitize (become sensitive) red blood cell.

(4) Control of complement

(5) Control of sensitivity of beginning of reaction while using positive serum

(6) Control of reaction specificity by using negative serum.

d) Calculation of results: Results in CFT test are realized according to international Standard anti Brucellosis Abortus Serum (USAbS). USAbS involves 1000 International Complement Fixation Unit (ICFTU) in 1 ml. It is standardized by Serum International Standard Serum (OIEISS) based. It is given as titer TUSAbS (OIEISS) when it is used in CFT. Titer of tested serum is given as TTESTSERUM. Test result is given by showing ICFTU of tested serum in each ml. Factor (F) which is used for calculation of ICFTU of gained titer is used to transform titer to ICFTU and also it is calculated according to following formula:

$$F = 1000 \times 1/ T \text{ USABS(OIEISS)}$$

The formula which is used to calculate international CFT unit (ICFTU testserum) in each ml of test serum is :

$$\text{ICFTU}_{\text{TESTSERUM}} = F \times 1/ T_{\text{USAbS}}$$

Example: Calculation of ICFTU of serum is giving positive  $T_{\text{TESTSERUM}}$  titer 1/320 and of serum is giving positive TUSABS titer 1/280 in applied CFT test;

$$F = 1000 \times 1/1/280 = 1000 \times 0.0078125 = 0.78125$$

$$\text{ICFTU}_{\text{TESTSERUM}} = 0.78125 \times 320 = 250$$

Sample serum includes 250 ICFTU. Titers which are evaluated as 20 ICFTU or more will be accepted as positive.

e) Interpreting of results; Serum which includes twenty or more ICFTU in each ml will be accepted as positive.

#### 6- Milk ring test (MRT)

a) Antigen phenol saline (NaCl %0,85 (m/v) and phenol %0,5 (v/v)) consists of painting bacterial suspension inside of it with hematoxylen. Antigen must be kept at 4C degree and not to be frozen.

b) Sensitivity of antigen is needed to be standardized by giving negative result in 1/1000 dilution while antigen OIEISS giving positive reaction in 1/500 dilution in relation with OIEISS.

c) Ring test must be applied at the samples which represent casting tank content or each milk churn content.

ç) Milk samples should not be frozen, heated or shaken excessively.

d) Reaction needs to be realized by using one of the following methods:

(1) Adding milk sample of 1ml volume to one of painted standardized antigens in the amount of either 0,03 ml or 0,05 ml at minimum 25mm height milk colon,

(2) Adding milk sample of 2 ml volume to one of painted standardized antigens in the amount of 0,05 ml at minimum 25mm height milk colon,

(3) Adding milk sample of 8ml volume to one of painted standardized antigens in the amount of 0,08.

e) Mixture of milk and antigen needs to be incubated at 37 C degree for sixty minutes with positive and negative working standards. After that, extra incubation will increase the sensitivity of the test at 4 C degrees from sixteen to twenty four hours period.

f) Interpreting the results;

(1) Negative reaction: colorful milk, colorless cream

(2) Positive reaction: exact same color milk and cream or colorful cream, colorless milk

#### 7- Rose Bengal Plate Test (RBT)

a) Antigen consists of buffered bacterial suspension within Brucellosis antigen diluents painted rose bengal at pH of  $3,65 \pm 0,05$ . Antigen which will be distributed as ready to use needs to be kept at 4 C degrees and not to be frozen.

b) Antigen should be prepared without considering cell concentration but it needs to be standardized to give negative result 1/55 dilution and positive result at 1/45 serum dilution related to OIEISS.

c) RBT needs to be done as follows:

(1) Serum (20-30 Jull) is mixed with equal volume of antigen at the glazing plate or at white ground (brick) and it is waited to create a zone in diameter of 2 cm. Mixture is shaken for four minutes period slowly and then it is analyzed by agglutination under the proper light.

(2) Automatic method can be used but it is obligatory that this method should be sensitive as at least manual method.

(3) Interpreting the results: As long as there is no extra drying at corners and sides, any considerable reaction will accepted as positive. Positive and negative working standards need to exist at each chain of test.

#### 8- Serum agglutination test (SAT).

a) Antigen phenol saline (NaCl %0,85 (m/v) and phenol %0,5 (v/v)) consists of bacterial suspension. Formaldehyde should be used. Delusion factor which is used for distributing of antigens in concentrate situation should be pointed at the bottle label. So distribution can be made. 5 mm of EDTA can be added to antigen suspension to decrease misleading positive proportion at serum agglutination test of final test delusion. Then pH at the antigen suspension can be adjusted to 7,2 again.

b) OIEISS 1000 includes international agglutination unit.

c) Antigen is prepared without considering cell concentration but its sensitivity needs to be standardized such that antigen should give either 50% agglutination at 1/600-1/1000 final serum dilution or give either 75% agglutination at 1/500-1/750 final serum dilution. Also it is recommended that reactivity of new or pre-standardized antigen guinea should not be mixed by using identified serum panel.

ç) Test is applied in either tubes or micro plates. Mixture of antigen and serum dilutions needs to be incubated at 37 C degree for sixteen to twenty four hours. There must be at least three dilutions for each serum. Suspicious serum dilutions need to be adjusted that they should be read at median tube (or micro plate method) in the limit of positivity.

d) Interpreting the results; Brucellosis agglutination degree of a serum is expressed from IU kinds for each ml. If it includes more than 30 IU, that serum will be considered as positive.

C- CELISA test

1- Competitive Enzyme Linked Immunosorbent Assay (cELISA) test.

2- Terms of use for cELISA;

a) cELISA can not be used for commercial oriented certificates.

b) Sample of cELISA is showed more specificity in comparison with indirect ELISA. Therefore, evaluation of serologic tests results can be used as a supportive method.

3- Test procedure will be realized in accordance with OIE Diagnostic Tests and Vaccinations Standards Hand Book.

Ç- National Reference Laboratories

1- International Reference Laboratories

2- Duties and Responsibilities of International Reference Laboratories

a) Approving validity exercises which shows the reliability of test method used in the country,

b) Determining the maximum number of samples which will create a ppol in used ELISA criteria,

c) Calibrating standard secondary national serums (work standards) to international primary standard serum,

ç) Quality controls of antigens which are used in country and ELISA kit beeches.

## **OFFICIAL BRUCELLOSIS (B. Melitensis) TESTS**

A- Tests

1- Rose Bengal Test;

a) Rose Bengal test can be used as screening test while gaining statue of pure from brucellosis or officially pure from brucellosis for sheep and goat herds. Application of test is done according to appendix 3.

b) While applying Rose Bengal test, if more than 5% of animals in business give positive reaction, there will be another test for each animals in business with complement fixation method.

2- Complement Fixation Method;

a) Complement Fixation test needs to be used for all isolated animal tests.

b) Complement Fixation test can be used for gaining statue of pure from brucellosis or officially pure from brucellosis for sheep and goat herds.

c) Serums which include 20 or more CFT unit/ml will be considered as positive.

3- Antigens in use must be approved by national reference laboratory. Secondary international standard needs to be standardized against to anti brucellosis abortus serum.

4- Working serum must be standardized with standard serum and it needs to be compatible with secondary international standard anti brucellosis abortus serum prepared by Central Veterinary Laboratory, Weybridge, Surrey, England.

#### B- CONTAGIOUS EPIDIDIMITIS (Brucellosis ovis) OFFICIAL TEST

##### 1- Complement fixing test

a) Specific antigen in use must be approved by international reference laboratory and be standardized against to international standard anti brucellosis ovis serum.

b) Working serum must be standardized with international standard anti brucellosis ovis serum prepared by Central Veterinary Laboratory, Weybridge, Surrey, England. Each serum which includes 50 or more international unit for each ml will be considered as positive.