Republic of Latvia

Cabinet
Regulation No. 63
Adopted 29 January 2013

Procedures for the Taking of Preventive and Eradication Measures of Brucellosis for Swine

Issued pursuant to
Section 25, Clause 4 of the Veterinary Medicine Law

1. General Provisions

1. This Regulation prescribes the procedures for preventive and eradication measures of brucellosis agent *Brucella suis* (hereinafter – brucellosis) in swine species (hereinafter – animal) (except animals living in the wild).

2. Terms used in this Regulation:

2.1 suspicions regarding infection with brucellosis – the animal has been in contact with an animal infected with brucellosis, or a potential source of brucellosis (e.g., the aborted foetus, amniotic fluid, placenta, stillborn or newborn dead animals, contaminated food or objects);

2.2 suspicions regarding illness with brucellosis – the animal displays clinically pathological signs characteristic to brucellosis and, upon determination of specific antibodies against the brucellosis agent in a blood serum sample of the animal, positive results of serological tests have been obtained in laboratory by using the Rose Bengal Test (hereinafter – RBT method) and the complement fixation test (hereinafter – CFT method);

2.3 animal infected with brucellosis – an animal with a confirmed diagnosis of brucellosis;

2.4 diagnosis of brucellosis – diagnosis, which is confirmed, if an animal displays clinically pathological signs characteristic to brucellosis and brucellosis agent has been obtained in laboratory bacteriological tests or positive results of serological tests have been obtained in laboratory upon determination of specific antibodies against the brucellosis agent by two methods, one of which is the CFT method and the other is the RBT method or agglutination test (hereinafter – the AT method);

2.5. official veterinarian – a practicing veterinarian authorised by the Food and Veterinary Service (hereinafter – Service) for implementation of measures for eradication of brucellosis;

2.6 affected area – a place (territory) where an animal with a confirmed diagnosis of brucellosis is kept and where measures for eradication of brucellosis are taken;

2.7 holding – a space, building, territory or part thereof, or a fenced area where animals are bred or kept, and they form one epidemiological unit;

2.8 control sample – a blood serum sample taken from an animal during preventive measures in order to carry out serological testing in a laboratory, determining the specific antibodies against the brucellosis agent;

2.9 clinical or pathological material sample – tissue samples taken from an animal or dead animal during measures for eradication of brucellosis – blood serum, aborted foetus, amniotic fluid, placenta, stillborn or newborn dead animals, reproductive organs;

2.10 status of a brucellosis-free holding – status granted by the Service and retained for a holding, if it has been officially brucellosis-free for nine months, and measures for the prevention and eradication of brucellosis are taken therein;

2.11 gilt – mature female or male swine before the first mating;

2.12 boar – a boar used to determine the female swine which are ready for mating.

3. The following is prohibited in the Republic of Latvia:

3.1 preventive vaccination of animals and use of hyperimmune serums against brucellosis;

3.2 therapeutic treatment of brucellosis for animals;

3.3 importation of such animals which have been vaccinated against brucellosis.

4. The Service shall:

4.1 draw up a programme for the monitoring and eradication of brucellosis;

4.2 in accordance with the Administrative Procedure Law take a decision:

4.2.1 to grant, renew or retain the status of an officially brucellosis-free holding for the holding if the animal owner or holder has taken the measures referred to in Paragraph 17, 18, 19, 23, 36, 37 or 52 of this Regulation;

4.2.2 to suspend or revoke the status of an officially brucellosis-free holding for the holding in accordance with Paragraph 27 or 36 of this Regulation.

5. If a holding has not been granted or retained the status of an officially brucellosis-free holding, the holding:

5.1 is prohibited to move animals (except the movement of animals to a slaughterhouse for immediate slaughter);

5.2 is prohibited to trade in animals and their products;

5.3 prior to mating, must ensure serological testing of blood serum samples of such gilt, which are older than six months;

5.4 is permitted to place gilt in the holding if, 30 days prior to placing gilt in the holding, the animals have been serologically tested.

6. Control samples shall be taken and submitted for laboratory testing to the State scientific institute “Institute of Food Safety, Animal Health and Environment “BIOR”” (hereinafter – Institute) by a practising veterinarian.

7. The animal owner or holder shall cover the costs of control sampling, transfer to the Institute and laboratory testing.

8. Samples of clinical and pathological material shall be taken and sent to the Institute by a practising veterinarian who has entered into an agreement with the Service regarding sampling of clinical or pathological materials, or an inspector of the Service, or an official veterinarian.

9. The Institute shall carry out:

9.1 serological testing, determining specific antibodies against the brucellosis agent. Testing shall be carried out using the following methods (Annex 1):

9.1.1 the RBT method in a blood serum sample;

9.1.2 the CFT method in a blood serum sample;

9.1.3 the AT method in a blood serum sample;

9.2 bacteriological testing, determining the brucellosis agent in a clinical or pathological material sample.

10. If the Institute carries out serological testing in an animal blood serum sample using the RBT or AT method and detects the presence of specific antibodies against the brucellosis agent, the sample shall be tested using the CFT method.

11. Upon carrying out of serological testing in an animal blood serum sample, the testing results shall be considered to be positive by the Institute, if, by using:

11.1 the RBT method, the presence of specific antibodies against the brucellosis agent is detected in the animal blood serum sample;

11.2 the AT method, at least 30 International Units of specific antibodies against the brucellosis agent are detected per millilitre of the animal blood serum sample;

11.3 the CFT method, at least 20 International Units of specific antibodies against the brucellosis agent are detected per millilitre of the animal blood serum sample.

12. After laboratory testing of the sample, the Institute shall send the testing results to:

12.1 the Agricultural Data Centre (hereinafter – Data Centre) – in electronic form;

12.2 the person who has sent in the sample – in electronic or paper form;

12.3 the Service and the respective territorial unit thereof in accordance with the regulatory enactment regarding infectious animal diseases subject to reporting, registration and State monitoring, and the procedures for informing the Food and Veterinary Service on such diseases – in electronic form, if positive result has been obtained.

13. The animal owner or holder and the Institute shall store the results of laboratory testing of brucellosis in electronic or paper form for at least three years after receipt thereof, but the Institute shall keep the isolated brucellosis agent for at least six months after its isolation in such a way that the brucellosis agent retains its biological properties.

14. The animal owner or holder who has not taken the preventive measures referred to in Chapter 2 of this Regulation shall cover all expenses related to the measures for eradication of brucellosis (expenses of an official veterinarian, the Service, the Institute and such persons who are involved in taking, processing and disposal of such by-products and derived products of animal origin which are not intended for human consumption (hereinafter – by-products)), if one of the following cases is established:

14.1 the diagnosis of brucellosis has been confirmed;

14.2 there are suspicions regarding illness with brucellosis;

14.3 there are suspicions regarding infection with brucellosis.

15. If there are suspicions regarding infection or illness with brucellosis, or the diagnosis of brucellosis has been confirmed and the animal owner or holder has taken the preventative measures referred to in Chapter 2 of this Regulation, the Ministry of Agriculture shall cover the following costs of measures for eradication of brucellosis from the financing assigned in the budget for the current year for the provision of epidemiological testing:

15.1. for an official veterinarian:

15.1.1 the transport costs;

15.1.2 work remuneration (for a visit of a veterinarian and drawing up of documents, for partial autopsy of a dead animal or aborted foetus, for taking and packaging of clinical or pathological material samples, for clinical testing of an animal and disinfection);

15.1.3 expenses related to the purchase of the utilised disinfection agents;

15.2 to the Institute – costs related to laboratory testing (serological (RBT, CFT and AT methods) and bacteriological testing).

16. The animal owner or holder shall collect, use, process or dispose of by-products in accordance with the instructions of the inspector of the Service, by applying processing methods and conditions laid down in Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (hereinafter – Regulation No 1069/2009) and Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive (hereinafter – Regulation No 142/2011)

2. Preventive Measures of Brucellosis

2.1 Measures to be Taken for a Holding to be Granted the Status of an Officially Brucellosis-free Holding

17. The animal owner or holder shall apply with the Service, by submitting an application in electronic or paper form for granting the status of an officially brucellosis-free holding to a holding (Annex 2). The application shall be accompanied by copies of laboratory test reports carried out for the detection of brucellosis during the last 12 months, if:

17.1 the Service does not have access to the necessary laboratory tests;

17.2 clinical characteristics of brucellosis have not been observed in animals over the last 9 months;

17.3 the conditions referred to in Paragraph 18 or 19 of this Regulation have been conformed to.

18. The holding where breeding boar or sow, or gilt and fattening pig are kept, shall conform to the following requirements with respect to the control sample testing:

18.1 a breeding boar is serologically tested once a year in accordance with the methods referred to in Sub-paragraph 9.1 of this Regulation, and the testing results are negative (hereinafter – serologically tested);

18.2 a boar and sow are serologically tested once every two years;

18.3 a gilt older than six months is serologically tested before mating;

18.4 fattening pig over the age of six months, not later than 30 days prior to insertion in the holding are serologically tested using the RBT method.

19. In the holding, in which only fattening pig are grown, the swine shall be brought from the holding, which has been granted the status of an officially brucellosis-free holding or the animals have undergone serological testing in accordance with Sub-paragraph 18.4 of this Regulation.

20. The Service shall assess the conformity of the holding with the criteria referred to in Paragraphs 17 and 18 of this Regulation or Paragraphs 17 and 19 of this Regulation, and shall take one of the following decisions:

20.1 to grant the status of an officially brucellosis-free holding to the holding, notifying the decision to the animal owner or holder in writing;

20.2 not to grant the status of an officially brucellosis-free holding to the holding, providing a justified refusal in writing.

21. If the Service decides to grant the status of an officially brucellosis-free holding, the granted status and the end date of the next required serological testing shall be recorded in the database of the Data Centre.

22. The Data Centre shall maintain a current list of the holdings on its website, which have been granted and retained the status of an officially brucellosis-free holding.

2.2 Measures to be Taken to Retain the Status of an Officially brucellosis-free Holding for a Holding

23. The Service shall retain the status of an officially brucellosis-free holding for the holding if:

23.1 the animal owner or holder ensures control sample testing for animals kept in the holding in accordance with the requirements referred to in Paragraph 24 of this Regulation;

23.2 the animals are placed in the holding in accordance with the procedures referred to in Paragraphs 25 and 26 of this Regulation.

24. In order for the Service to retain the status of an officially brucellosis-free holding for the holding, the animal owner or holder shall ensure that once a year the following is ensured:

24.1 serological testing of breeding boar blood serum sample;

24.2 serological testing of blood serum samples of 10 per cent of such sows, which were not tested in the previous year.

25. The animal owner or holder may place animals in the holding, which has been granted the status of an officially brucellosis-free holding:

25.1 from the holding of the same status, and a breeding boar or sow may be placed if the following control sample testing has been performed:

25.1.1 a breeding boar has been serologically tested not later than 30 days prior to placing in the holding;

25.1.2 a sow is serologically tested once a year;

25.2 from a European Union Member State or a third country in accordance with the laws and regulations regarding the veterinary requirements for bovine and swine circulation and the veterinary requirements for circulation of such animals, which is not referred to in other laws and regulations regarding veterinary control, as well as in accordance with Commission Regulation (EU) No 206/2010 of 12 March 2010 laying down lists of third countries, territories or parts thereof authorised for the introduction into the European Union of certain animals and fresh meat and the veterinary certification requirements.

26. If animals which have not been serologically tested prior to placement in the holding are imported from another European Union Member State or third country, the animal owner or holder shall isolate the animals, carry out serological testing and, if the results are negative, place them in the holding with other animals.

27. The Service shall revoke the status of an officially brucellosis-free holding from the holding if it detects one of the following cases:

27.1 the animal owner or holder places animals from the holding with the status of an officially brucellosis-free holding in the holding which has been revoked the status of an officially brucellosis-free holding;

27.2 animals have not been registered in the database of the Data Centre according to the actual situation in the holding;

27.3 the conditions referred to in Paragraphs 24, 25 and 26 of this Regulation are not being fulfilled;

27.4 a positive result of laboratory testing using the RBT, AT or CFT method has been received;

27.5 an official veterinarian or an inspector of the Service has confirmed the diagnosis of brucellosis.

3. Measures for Eradication of Brucellosis

3.1 Measures to be Taken if There are Suspicions Regarding Infection or Illness with Brucellosis

28. The animal, which is suspected of being infected or ill with brucellosis, shall labelled and kept isolated from other animals in the holding, protecting them from infection with brucellosis.

29. It is prohibited to move animals outside the holding until the determination of diagnosis, except movement of animals to a slaughterhouse for immediate slaughtering, in accordance with the conditions referred to in Paragraph 46 of this Regulation.

30. The animal owner or holder, using any means of communication, within 24 hours shall inform the practicing veterinarian or the relevant territorial unit of the Service regarding the animal, which had an abortion, as well as if there are suspicions regarding infection or illness with brucellosis.

31. If a practicing veterinarian is not authorised to take measures for eradication of brucellosis, after receipt of the information referred to in Paragraph 30 of this Regulation he or she shall notify the relevant territorial unit of the Service without delay.

32. The territorial unit of the Service shall, within 24 hours after receipt of the information referred to in Paragraph 30 of this Regulation, using any means of communication (in writing, in electronic form or in oral form):

32.1 inform the animal owner or holder regarding the official veterinarian or inspector of the Service, who will take the measures for eradication of brucellosis;

32.2 hand over this information to the official veterinarian or inspector of the Service.

33. When initiating ascertaining of the diagnosis of brucellosis, the official veterinarian or inspector of the Service shall, within 24 hours, clinically examine the animal at the site where the animal is kept, assess the epizootiological situation and check whether the necessary control sample testing has been carried out.

34. The official veterinarian or inspector of the Service, after performing the actions referred to in Paragraph 33 of this Regulation, shall take the clinical or pathological material sample and send it for laboratory testing to the Institute for determination of the diagnosis of brucellosis.

35. In order to take a pathological material sample for bacteriological testing, the animal, which is suspected to be infected with brucellosis, shall be killed, if necessary. The animal owner or holder shall use, process or dispose of the killed animal in accordance with the instructions of the inspector of the Service, applying processing methods and conditions laid down in Regulation No 1069/2009 and Regulation No 142/2011.

36. The Service shall suspend the status of an officially brucellosis-free holding for the respective holding upon receiving a positive result of laboratory testing using the RBT and

CFT method or the AT and CFT method. The referred-to status may be renewed if the animal owner or holder ensures that within 30 days:

36.1 the serologically positive animal is re-tested serologically using the CFT method and, if a pathological material sample is available, it is bacteriological tested and the diagnosis of brucellosis is not confirmed;

36.2 animals which are more than six months old and which have been in contact with an animal suspected of being infected or ill with brucellosis, shall be serologically tested with the RBT method;

36.3 random testing of 30 per cent of sows and all boars is performed.

37. If the serologically positive animal has been slaughtered during measures for eradication of brucellosis and repeated sampling is not possible, the status of an officially brucellosis-free holding shall be renewed for the holding, if all animals over six months of age have been serologically tested twice using the RBT method. The first serological testing shall be carried out 30 days after slaughtering (moment of isolation) of serologically positive animals and the second testing - 60 days after the first testing by using the RBT method.

3.2 Measures to be Taken if There is a Confirmed Diagnosis of Brucellosis

38. It is prohibited to move animals outside the affected area while taking the measures for eradication of brucellosis (hereinafter – limitation period), except movement of animals to a slaughterhouse for an immediate slaughtering, in accordance with the conditions referred to in Paragraph 46 of this Regulation.

39. Upon receipt of information regarding confirmed diagnosis of brucellosis, the relevant territorial unit of the Service shall within one working day:

39.1 determine and approve the plan of measures for eradication of brucellosis in accordance with Paragraph 41 of this Regulation;

39.2 determine the brucellosis affected area in accordance with Paragraph 42 of this Regulation;

39.3 perform epizootiologial case studies.

40. The territorial unit of the Service shall inform regarding the measures referred to in Sub-paragraphs 39.1 and 39.2 of this Regulation within two working days after taking thereof:

40.1 the relevant local government;

40.2 the official veterinarian;

40.3 the Centre for Disease Prevention and Control.

41. The plan of measures for eradication of brucellosis shall lay down the measures for eradication of brucellosis in the affected area, indicating the sequence of eradication measures, the person responsible for control of the measures for eradication of brucellosis, epidemiological testing and handling of animals, fresh meat, slaughter products and by-products, as well as the limitation period and other measures.

42. The Service shall determine the affected area taking into account the following:

42.1 the epizootiological situation research results in the given area and the identification of the place of origin of the brucellosis agent, pathways and possible infected objects;

42.2 geographical and administrative boundaries;

42.3 social factors and density of animal population.

43. After carrying out the activities referred to in Paragraph 39 of this Regulation, the Service shall:

43.1 within one working day, post information regarding the approved case of brucellosis on the website, indicating:

43.1.1 the date when the diagnosis of brucellosis was confirmed;

43.1.2 the group of animals (breeding boar, sow, gilt, piglet, fattening pig);

43.1.3 the number of animals;

43.1.4 the brucellosis affected area;

43.1.5 the minimum limitation period;

43.2 report to the European Commission, other European Union Member States and the World Organisation for Animal Health on the approved diagnosis of brucellosis in accordance with the regulatory enactment regarding infectious animal diseases subject to reporting, registration and State monitoring, and the procedures for informing the Food and Veterinary Service on such diseases.

44. The animal owner or holder shall, within six months after confirmation of the diagnosis of brucellosis, upon receipt of the bacteriological testing results referred to in Paragraph 9 of this Regulation or repeated positive result of serological testing with the RBT, CFT or AT method, ensure that the following is killed in the affected area:

44.1 animals infected with brucellosis;

44.2 animals which are suspected to be infected with brucellosis;

44.3 piglets which are not weaned from the sow.

45. The animal owner or holder shall use, process or dispose of carcasses of the killed animals referred to in Paragraph 44 of this Regulation in accordance with the instructions of the inspector of the Service, applying the processing methods and conditions laid down in Regulation No 1069/2009 and Regulation No 142/2011.

46. In the case of confirmed diagnosis of brucellosis the Service shall revoke the status of an officially brucellosis-free holding and, on the basis of the epizootiological situation research results, decide on one of the following actions, carrying out of which shall be provided in the affected area by the animal owner or holder:

46.1 killing of piglets weaned from a sow in the affected area and use, processing or disposal of their carcasses in accordance with the instructions of an inspector of the Service, applying the processing methods and conditions laid down in Regulation No 1069/2009 and Regulation No 142/2011, or their dispatching for slaughtering to a slaughterhouse, as well as sending of an animal, which is suspected of being infected with brucellosis, and all animals susceptible to brucellosis to a slaughterhouse;

46.2 sending of piglets weaned from a sow to a slaughterhouse or isolation from other animals kept in a holding and taking of clinical or pathological material sample from them when they are six months old and serological testing with the CFT method twice with an interval not less than two months, and serological testing with the CFT method of the animals, which are suspected of being infected with brucellosis, and animals susceptible to brucellosis, with an interval not less than two months.

47. The serological testing referred to in Sub-paragraph 46.2 of this Regulation shall be continued until all animals kept in a holding have negative results in two repeated serological tests with the CFT method. If positive results are obtained, the animals shall be sent for slaughtering to a slaughterhouse.

48. The animals referred to in Paragraph 46 of this Regulation, after slaughtering at a slaughterhouse, may be used for the production of food or animal feed, performing such treatment of slaughter by-products to eliminate brucellosis agent.

49. After carrying out the activities referred to in Paragraph 44 and Sub-paragraph 46.1 or 46.2 of this Regulation the animal owner or holder under the supervision of an inspector of the Service or an official veterinarian shall ensure the destruction of the brucellosis agent:

49.1 in accordance with the processing methods and conditions laid down in Regulation No 1069/2009 and Regulation No 142/2011 by processing or recycling manure, which has been in contact with serologically positive animals or sources of the brucellosis agent (placenta, aborted foetus, embryo, stillborn or dead piglets);

49.2 by cleaning and washing the holding, equipment, inventory of the holding and vehicles, which have been used for transportation of susceptible animals, as well as by disinfecting the referred-to places and objects with a disinfectant that destroys the brucellosis agent.

50. After disinfection of the holding an inspector of the Service shall take disinfection quality control samples and send them to the Institute.

51. Outdoor enclosures in the area affected by brucellosis shall be used to no earlier than 60 days after carrying out of the activities referred to in Paragraph 49 of this Regulation.

52. The Service shall renew the status of an officially brucellosis-free holding for the holding after the end of the measures for eradication of brucellosis if the animal owner or holder:

52.1 has complied with the requirements referred to in Paragraph 44 and Sub-paragraph 46.1 or 46.2 of this Regulation, as well as in Paragraphs 47 and 49 of this Regulation;

52.2 has placed animals in the holding in accordance with the conditions referred to in Paragraph 25 or 26 of this Regulation six months after revocation of restrictions in the affected area;

52.3. has submitted to the Service, in electronic or paper form, an application for granting the status of an officially brucellosis-free holding (Annex 2), appending copies of test reports on laboratory testing carried out over the last 12 months in the holding for determination of brucellosis, if the Service does not have access to the necessary laboratory tests.

53. In the holdings, which are epizootiologically related to the affected area, the Service shall determine:

53.1 random carrying out of the necessary control sample testing for 25 per cent of all susceptible animals;

53.2 a prohibition on movement and slaughtering of animals until serological testing is carried out on the animals referred to in Sub-paragraph 53.1 of this Regulation.

54. The territorial unit of the Service shall prohibit organising of animal exhibitions, races and other events with the participation of animals at the affected area.

55. The Centre for Disease Prevention and Control shall ensure epidemiological testing of the brucellosis incident and organisation of anti-epidemic measures, as well as organise laboratory testing and medical follow-up of exposed persons in accordance with the laws and regulations regarding the procedures for the determination of exposed persons, initial medical examination, laboratory examination and medical observation.

56. The official veterinarian shall inform the relevant territorial unit of the Service within two working days after implementation of the plan of measures for eradication of brucellosis in writing.

57. The territorial unit of the Service, after evaluation of the information referred to in Paragraph 56 of this Regulation, shall decide on retaining or revocation of the status of the affected area.

58. If the territorial unit of the Service decides to revoke the status of the affected area, the Service shall, within two working days after taking of the decision, inform the Centre for Disease Prevention and Control thereof.

Informative Reference to the European Union Directive

This Regulation contains legal norms arising from Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals and swine.

Prime Minister V. Dombrovskis

Acting for the Minister for Agriculture –
Minister for Health I. Circene

Annex 1
Cabinet Regulation No. 63
29 January 2013

Description of the Methods of Laboratory Diagnostics of Swine Brucellosis

1. Identification of Disease-Causing Agents

1. If, by using a modified acid-fast or immunospecific staining of organisms, the morphology of *Brucella spp*. is detected in abortion material, vaginal discharges or milk, it shall cause grounds for suspicion that the animal is infected with brucellosis, especially if supported by serological testing methods. Polymerase chain reaction may be used as an additional method for the detection of swine brucellosis agent.

2. If possible, *Brucella spp*. shall be isolated by using a simple or selective culture medium with cultivation from vaginal discharges, aborted foetuses, udder secretions or other selected tissues, for example, lymph nodes, male and female reproductive organs.

3. After isolation, the species and biological varieties of *Brucella spp.* shall be determined by phage lysis or oxidative metabolism test in accordance with cultivation, biochemical and serological criteria. Polymerase chain reaction is a method that enables to determine the biotype based on specific genetic combinations.

4. The standardisation of the laboratory methods used and the culture media, as well as the interpretation of results shall be performed in accordance with the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the World Organisation for Animal Health (hereinafter - OIE), the current version in Latvian for swine brucellosis is available on the website of State scientific institute "Institute of Food Safety, Animal Health and Environment "BIOR"".

2. Immunological Methods

2.1 Reference Materials

5. *Brucella abortus* biovar 1, Weybridge strain No 99 or USDA strain 1119-3 shall be used for the preparation of all those antigens that are used for testing of blood serum sample by means of Rose Bengal Test (hereinafter – RBT method), agglutination test (hereinafter - AT method) and complement fixation test (hereinafter – CFT method).

6. The standard reference serum for the RBT, AT and CFT method is OIE international reference standard serum (hereinafter – OIEISS).

7. OIEISS is primary international standard serum, from which by the State reference laboratory establishes the national secondary reference standard serum (hereinafter – working standard serum) according to the methods referred to in Paragraph 5 of this Annex.

2.2 Complement Fixation Test

8. The antigen represents a bacterial suspension in phenol-saline (NaCl 0.85% (m/v) and phenol at 0.5% (v/v)) or in a veronal buffer solution. Antigens may be delivered in the concentrated state provided the dilution factor to be used is indicated on the package. The antigen must be stored at the temperature of 4°C and not frozen.

9. Blood serum of swine must be inactivated 30 to 50 minutes at the temperature of 60°C.

10. In order to carry out the test in accordance with CFT method, the complement dose higher than the minimum necessary for total haemolysis shall be used.

11. Each time, when the CFT is carried out, the following controls shall be made:
- 11.1 control of the anti-complementary effect of the blood serum;
- 11.2 control of the antigen;
- 11.3 control of sensitised red blood cells;
- 11.4 control of the complement;
- 11.5 control using a positive serum of sensitivity at the start of the reaction;
- 11.6 control of the specificity of the reaction, using a negative serum.

12. The OIEISS contains 1000 international specific complement fixation test units of antibody (hereinafter – ICFTU) per millilitre.

13. If the OIEISS is tested in the CFT method, the result is given as a titre (i.e., the largest direct OIEISS dilution that ensures 50% haemolysis, T_{OIEISS}). The CFT result for the blood serum sample given as a titre ($T_{TESTSERUM}$) must be expressed in ICFTU per millilitre. In order to convert the expression of a titre into ICFTU, the factor (F) necessary to convert a titre of an unknown CFT blood serum ($T_{TESTSERUM}$) tested by that method into the ICFTU expression can be found from the formula:

$$F = 1000 \times 1 / T_{OIEISS}$$

The content of international specific complement fixation test units of antibody per millilitre of blood serum sample ($ICFTU_{TESTSERUM}$) can be found from the formula:
$ICFTU_{TESTSERUM} = F \times T_{TESTSERUM}$

14. Interpretation of the results achieved by the CFT method - testing result is considered to be positive, if at least 20 or more ICFTU to the swine brucellosis agent are detected per millilitre of a blood serum sample.

2.3 Rose Bengal Reaction

15. The antigen represents a bacterial suspension in buffered solution with a pH 3.65 ± 0.05, stained by the use of Rose Bengal dye. The antigen shall be delivered ready for use and must be stored at the temperature of 4°C and not frozen.

16. The antigen shall be prepared without reference to the cell concentration, but its sensitivity must be standardised in relation to the OIEISS in such a way that the antigen produces a positive reaction in a blood serum dilution of 1:45 and a negative reaction with a dilution of 1:55.

17. Conditions for the use of the RBT method:

17.1 blood serum (20–30 µl) is mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone of approximately 2 cm in diameter. The mixture is rocked gently for 4 minutes at ambient temperature, and then observed in a good light for agglutination;

17.2 an automated method may be used but must be at least as sensitive and accurate as the manual method.

18. Interpretation of the results achieved by the RBT method – any visible reaction is considered to be positive, unless there has been excessive drying round the edges. Positive and negative working standards should be included in each series of RBT tests.

2.4 Agglutination Test

19. The antigen represents a bacterial suspension in phenol-saline (NaCl 0.85% (m/v) and phenol at 0.5% (v/v)). Formaldehyde may not be used. Antigens may be delivered in the concentrated state provided the dilution factor to be used is indicated on the bottle label. When testing a blood serum sample with the AT method, EDTA may be added to the antigen suspension up to 5 mM final test dilution in order to reduce the risk of false positives. pH of 7.2 must be readjusted to the antigen suspension.

20. The OIEISS contains 1000 International Units of agglutination.

21. The antigen shall be prepared without reference to the bacterial cell concentration, but its sensitivity must be standardised in relation to the OIEISS in such a way that the antigen produces either a 50% agglutination with a final blood serum dilution of 1:600 to 1:1000 or a 75% agglutination with a final blood serum dilution of 1:500 up to 1:750. It may also be advisable to compare the reactivity of new and previously standardised batches of antigen using a panel of defined sera.

22. Testing by means of the AT method shall be performed either in tubes or in microplates. The mixture of antigen and blood serum dilutions should be incubated for 16 to 24 hours at the temperature of 37°C. At least three dilutions are prepared for each blood serum. Dilutions of suspect blood serum must be made in such a way that the reading of the reaction at the positivity limit in the median tube (or well for the microplate method).

23. Interpretation of the results achieved with the AT method – the degree of agglutination in a blood serum must be expressed in International Units per millilitre (hereinafter – IU/ml). Test result shall be considered to be positive, if at least 30 or more IU/ml against the swine brucellosis agent are detected in the blood serum sample.

3. Tasks and Duties of the State Reference Laboratory.

24. State reference laboratories shall be responsible for:

24.1 approval of those research results, which prove the credibility of laboratory method used in the Member State;

24.2 calibration of working standard serum in relation to the primary international standard serum referred to in Sub-chapter 2.1 of this Annex.

24.3 quality checks for all antigen;

24.4 co-operation with the reference laboratories of the Member States of the European Union in relation to the diagnosis of brucellosis.

Acting for the Minister for Agriculture -
Minister for Health I. Circene

Annex 2
Cabinet Regulation No. 63
29 January 2013

Application for the Granting of the Status of an Officially Brucellosis-free Holding

1. Given name and surname or name of a legal person (capital letters)

_____

_____

2. Address of the declared place of residence of a natural person or legal address of a legal person (capital letters)

_____

_____

_____

_____

3. Contact details:

3.1. telephone number or mobile phone number

_____

3.2. fax number

_____

3.3. e-mail address

_____

4. Registration number in the Commercial Register or the Enterprise Register

L	V										

5. Registration number of the herd in the register of the Agricultural Data Centre

L	V										

6. Registration number of the holding in the register of the Agricultural Data Centre*

L	V										

7. Address of the actual location of the holding intended for granting of the status

_____

_____

_____

_____

8. At the holding:
8.1 clinical symptoms of brucellosis _____ observed in animals over last nine months;
(have/have not)

8.2 the following animals are kept (indicate the number by animal group – boars, sows, gilts, piglets and fattening pig)

8.3 animals have been serologically tested during the last 12 months (testing review number)**

(date***)

(given name, surname) (signature***)

Notes.
1. * Also indicate the internal number of the holding assigned by the herd owner or holder, if such has been granted.
2 ** Append copies of testing reviews.
3. *** The details of the document "date" and "signature" need not be completed if the electronic document has been prepared in accordance with the laws and regulations regarding the drawing up of electronic documents.

Acting for the Minister for Agriculture -
Minister for Health I. Circene