Republic of Latvia

Cabinet
Regulation No. 881
Adopted 18 December 2012

Procedures for the Taking of Prevention and Eradication Measures of Brucellosis in Bovine Animals

Issued in accordance with
Section 25, Clause 4 of
the Veterinary Medicine Law

1. General Provisions

1. This Regulation prescribes the procedures for the prevention and eradication of *Brucella abortus* agent (hereinafter – brucellosis), which causes brucellosis in bovine animals (hereinafter – animals).

2. Terms used in this Regulation:

2.1. 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 the sample collected from the respective animal, positive results of serological tests have been obtained by using the Rose Bengal Test (hereinafter – RBT method) and the complement fixation test (hereinafter – CFT method);

2.2. animal infected with brucellosis – an animal with a confirmed diagnosis of brucellosis;

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

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

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

2.6. sample of clinical or pathological material – tissue samples taken from an animal or dead animal during eradication measures of brucellosis – blood serum, aborted foetus, amniotic fluid, placenta, carcass of a stillborn or newborn animal, uterine discharges, lymph nodes, male or female reproductive organs;

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

2.8. undefined brucellosis status – status granted by the Service and retained for a holding, if the health status of animals, the results of laboratory tests or vaccination measures against brucellosis are not known on the day of determining the status.

2.9. defined brucellosis status – status granted by the Service and retained for a holding, if the health status of animals, the results of laboratory tests and vaccination measures against brucellosis are known, serological testing of animals is performed and the holding qualifies for the status of a brucellosis-free or an officially brucellosis-free holding;

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

2.11. status of an officially brucellosis-free holding – status granted by the Service and retained for a holding, if it has kept the status of a brucellosis-free holding for two years and measures for the prevention and eradication of brucellosis are taken therein;

2.12. status of an officially brucellosis-free country – status granted by the European Commission and retained for a country, if the diagnosis of brucellosis has not been confirmed in the respective country for three years and 99.8 per cent of animal holdings have had the status of officially brucellosis-free holdings for five consecutive years, all animals are identified and the Service provides information regarding the situation in the country regarding the confirmed cases of brucellosis, as well as if the measures for prevention and eradication of brucellosis are taken;

2.13. means of transport – those parts of motor vehicles, rail vehicles and aircraft set aside for loading, the holds of ships and containers for land, sea or air transport.

3. The following is prohibited in the Republic of Latvia (hereinafter – Latvia):

3.1. preventive vaccination of animals and use of hyperimmune serums against brucellosis, except the cases referred to in Sub-paragraphs 31.3 and 38.1 of this Regulation;

3.2. importation of animals that have been vaccinated against brucellosis, except the cases where the conditions referred to in Sub-paragraphs 41.3.1 and 41.3.3 of this Regulation are met;

3.3. therapeutic treatment of brucellosis.

4. The Service shall carry out the necessary activities for retaining the status of Latvia as an officially brucellosis-free country in accordance with Paragraph 5 of this Regulation.

5. The Service shall:

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

5.2. until 31 May of the current year provide information to the European Commission regarding the situation in the previous year in relation to the brucellosis agent.

6. The Service shall take decisions on the following in accordance with the procedures laid down in the Administrative Procedure Law:

6.1. to grant or to retain the status of a brucellosis-free holding or the status of an officially brucellosis-free holding, if the animal owner or holder has carried out the measures for prevention and eradication of brucellosis;

6.2. to cancel or to suspend the status of a brucellosis-free holding or the status of an officially brucellosis-free holding, if suspicions regarding infection or illness of an animal with brucellosis are detected at the holding.

7. In order to carry out the necessary testing of control samples for animals in the following year, the Service shall submit information to the Agricultural Data Centre (hereinafter – Data Centre) regarding the necessary number of control samples and their selection criteria until 1 September of the current year.

8. The Data Centre shall prepare by 31 October of the current year and post on its website information for animal owners or holders and the Service regarding the holdings registered in the animal register, where the tests of control samples are required in the following year.

9. Control samples shall be taken and sent for laboratory testing to the State scientific institute "Institute of Food Safety, Animal Health and Environment "BIOR"" (hereinafter – Institute) by a practicing veterinarian.

10. The animal owner or holder shall cover expenses for taking of control samples, sending and laboratory testing thereof.

11. Samples of clinical or pathological material shall be taken and sent to the Institute by a practicing veterinarian who has signed a contract with the Service regarding taking of clinical or pathological material samples, or by an inspector of the Service, or an official veterinarian.

12. The Institute shall carry out:

12.1. serological testing, determining specific antibodies against the brucellosis agent by using the following methods (Annex 1):

12.1.1. RBT method – in a blood serum sample;

12.1.2. CFT method – in a blood serum sample;

12.1.3. agglutination test – in a blood serum sample (hereinafter – AT method);

12.1.4. immunoenzymatic tests – in a blood serum sample or an individual, mixed or common milk sample (hereinafter – ELISA method);

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

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

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

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

14.2. 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;

14.3. 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.

15. If the status of an officially brucellosis-free country is granted to Latvia, the diagnosis of brucellosis in animals may be confirmed, if the following results have been acquired by means of laboratory methods:

15.1. positive result of serological testing, determining specific antibodies against the brucellosis agent by using two methods, if one of the methods is the CFT method;

15.2. brucellosis agent by means of bacteriological tests.

16. If the status of an officially brucellosis-free country is suspended for Latvia, the diagnosis of brucellosis in animals may be confirmed, if positive results of serological testing by determining specific antibodies against the brucellosis agent have been obtained:

16.1. in accordance with Paragraphs 79, 80 and 82 of this Regulation, if the holding has the status of a brucellosis-free holding;

16.2. in accordance with Paragraphs 79 and 80 of this Regulation, if the holding has the status of an officially brucellosis-free holding.

17. After laboratory testing of the sample, the Institute shall send the testing results within one working day:

17.1. to the Data Centre – in electronic form;

17.2. to the person who sent in the sample – in electronic or paper form;

17.3. to 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.

18. The animal owner or holder and the Institute shall store the results of laboratory testing of brucellosis 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.

19. In order for the Service to grant or retain the respective status of the holding, the animal owner or holder shall take the preventive measures referred to in Chapter 2 of this Regulation. Until carrying out thereof:

19.1. moving of animals, as well as trade in animals and their products is prohibited;

19.2. the Service shall not grant or suspend the status granted to the holding.

20. 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 eradication measures 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:

20.1. the diagnosis of brucellosis has been confirmed in accordance with Paragraph 15 or 16 of this Regulation;

20.2. there are suspicions regarding illness with brucellosis;

20.3. there are suspicions that an animal is infected with the brucellosis agent (hereinafter – suspicions regarding infection with brucellosis), which is manifested in one of the following ways:

20.3.1. the animal has been in contact with an animal suffering from brucellosis or a potential source of origin of brucellosis (for example, aborted foetus, amniotic fluid, placenta, stillborn or newborn dead animal, contaminated food or object);

20.3.2. clinical symptoms characteristic to brucellosis are observed in the animal – movement disorders, abscesses, bursitis, tendinitis, arthritis, paralysis of the backside of the body, for male animals – orchitis, signs of epididymitis, for female animals – miscarriage, inflammation of genitals, signs of mastitis, infertility;

20.3.3. positive result of serological laboratory testing has been obtained upon determining the specific antibodies against the brucellosis agent by using the RBT and CFT methods.

21. If there are suspicions that the animal has been infected or is ill with brucellosis, or diagnosis of brucellosis has been confirmed and the animal owner or holder has taken the preventive measures referred to in Chapter 2 of this Regulation, the Ministry of Agriculture shall cover the following costs of eradication measures of brucellosis from the financing assigned in the budget for the current year for the prevention and eradication of brucellosis:

21.1. for an official veterinarian:

21.1.1. the actual transport costs;

21.1.2. work remuneration (for a visit of a veterinarian and drawing up of documents, for partial autopsy of dead animal or aborted foetus, for taking and

packaging of clinical or pathological material samples, for clinical testing of an animal and disinfection);

21.1.3. expenses related to the purchase of disinfection agents;

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

22. If diagnosis of brucellosis has been confirmed, the losses resulting during eradication of brucellosis shall be covered to the animal owner in accordance with the regulatory enactment regarding the procedures for the granting and receipt of compensations by the animal owner for the losses resulting during an outbreak of State monitored animal disease or epizootic disease, if the animal owner or holder has taken preventive measures in order for a holding to be granted the respective status or to retain the respective status.

23. 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 (EC) 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).

24. The Service shall be responsible for the provision of information to the European Commission and other Member States of the European Union 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 regarding such diseases.

25. If the diagnosis of brucellosis is confirmed, the Service shall additionally provide information to the European Commission regarding:

25.1. the percentage and quantitative amount of holdings subject to eradication measures and the number of holdings where the diagnosis of brucellosis has been confirmed;

25.2. the total number of animals in the following categories:

25.2.1. animals subject to eradication measures;

25.2.2. animals, regarding which there are suspicions that they have been infected or are ill with brucellosis;

25.2.3. animals infected with brucellosis;

25.2.4. slaughtered animals;

25.3. the plan of eradication measures of brucellosis and the duration of implementation thereof;

25.4. the method used for the efficiency control of implementation of the plan of eradication measures of brucellosis;

25.5. funds from the State budget assigned for eradication of brucellosis and distribution of these funds according to positions.

26. The Service shall provide the information referred to in Sub-paragraphs 25.1, 25.2 and 25.3 of this Regulation regarding three years preceding the application of the plan of eradication measures of brucellosis and afterwards – every year until the plan of eradication measures of brucellosis is implemented.

2. Preventive Measures of Brucellosis

2.1. Measures to be Taken for a Holding to be Granted and Retained the Status of an Officially Brucellosis-free Holding if Latvia has been Granted the Status of an Officially Brucellosis-free Country

27. The status of an officially brucellosis-free holding shall be retained for a holding, if the animal owner or holder:

27.1. ensures testing of the control samples referred to in Paragraph 28 of this Regulation;

27.2. animals are placed in the holding in accordance with Paragraph 41 of this Regulation.

28. During the first five months after receipt of the status of an officially brucellosis-free country the Service shall determine the mandatory testing of control samples in a way that would once a year enable serological testing of 20 per cent of the holdings where animals are older than 24 months, using any of the methods laid down in Sub-paragraph 12.1 of this Regulation. In the following years the animal owner or holder shall carry out and ensure only eradication measures in accordance with Sub-chapter 3.1 of this Regulation.

29. The status of Latvia as an officially brucellosis-free country shall be retained, if the results of the serological testing referred to in Paragraph 28 of this Regulation are negative (hereinafter – serologically tested).

2.2. Measures to be Taken for a Holding to be Granted and Retained the Status of a Brucellosis-free Holding, if the Status of an Officially Brucellosis-free Country has Been Suspended for Latvia

2.2.1. Granting of the Status of a Brucellosis-free Holding

30. If the status of an officially brucellosis-free country has been suspended for Latvia and:

30.1. the status of a brucellosis-free holding has been suspended for the holding, the animal owner or holder may apply to the Service for granting of the status of a brucellosis-free holding (Annex 2), provided that it has taken the eradication measures of brucellosis laid down in Sub-chapter 3.2 of this Regulation and complied with the requirements referred to in Paragraph 31.

30.2. the status of a brucellosis-free holding has been previously retained for the holding, the animal owner or holder shall implement and ensure the preventive measures referred to in Sub-chapter 2.2.2 of this Regulation. In such case the status of a brucellosis-free holding shall be retained for the holding in future as well.

31. The Service shall grant the status of a brucellosis-free holding to a holding within one month after receipt of the application (Annex 2), if at the holding:

31.1. clinical symptoms characteristic to brucellosis have not been observed in animals during last six months;

31.2. animals that are older than 12 months have been serologically tested by selecting one of the following testing schemes:

31.2.1. blood serum samples of animals are serologically tested twice with an interval that is not less than three months and not more than 12 months;

31.2.2. milk samples of animals are serologically tested by using the ELISA method three times a year with an interval of at least three months and blood serum

samples of animals are serologically tested not earlier than six weeks after testing of the third milk sample;

31.3. female animals until six months of age have been vaccinated against brucellosis with Strain 19 live vaccine or until 15 months of age – with inactivated 45/20 auxiliary vaccine;

31.4. animals less than 30 months of age, which have been vaccinated with Strain 19 live vaccine, have been serologically tested, using one of the following methods:

31.4.1. the AT method, and its result shall be considered negative, if 30 to 80 International Units of specific antibodies against the brucellosis agent are detected per millilitre of the animal serum sample tested;

31.4.2. the CFT method, and its result shall be considered negative, if less than 30 international complement-fixation test (hereinafter – CFT) units against the brucellosis agent per millilitre of the blood serum sample taken from the female animal tested are detected and if vaccination against brucellosis in these animals has been performed within 12 months before testing, or less than 20 international CFT units against the brucellosis agent per millilitre, if vaccination against brucellosis in animals has been performed more than one year before testing.

32. The Service has the right to determine the necessity to vaccinate the animals referred to in Sub-paragraph 31.3 or 38.1 of this Regulation in the country in accordance with the requirements laid down in the regulatory enactment regarding the labelling, distribution and control of veterinary medicinal products.

2.2.2. Retaining of the Status of a Brucellosis-free Holding

33. The status of a brucellosis-free holding shall be retained for a holding, if at the holding:

33.1. each year control samples are taken from animals in accordance with the requirements referred to in Paragraph 34 of this Regulation and animals are serologically tested;

33.2. the animals are stationed in accordance with Paragraph 35 of this Regulation.

34. Upon granting the status of a brucellosis-free holding, the Service shall determine testing of control samples to be performed for animals each year, selecting one of the following testing schemes:

34.1. milk samples of animals are tested, using the ELISA method with an interval of at least three months;

34.2. milk samples of animals are serologically tested, using the ELISA method twice with an interval of at least three months and blood serum samples of animals are serologically tested not earlier than six weeks after testing of the second milk sample;

34.3. blood serum samples of animals are serologically tested twice with an interval that is not less than three months and not longer than 12 months.

35. The animal owner or holder may station animals at an animal holding, which has been granted the status of a brucellosis-free holding, in one of the following cases:

35.1. if animals, which are over 12 months of age and which are from a holding that has been granted the status of an officially brucellosis-free holding, have been serologically tested (blood serum samples with the AT method) not later than 30 days after stationing thereof at the holding and negative results have been received, or if they have been serologically tested, using any other of the methods referred to in Sub-paragraph 12.1 of this Regulation. If serological testing of animals has not been performed before stationing of such animals at the holding, the animals shall be isolated and stationed with other animals at the

holding only after they have been subject to serological testing within 30 days after their stationing at the holding;

35.2. the animals referred to in Sub-paragraph 35.1 of this Regulation need not be subject to serological testing, if they are moved from a country or an administrative territory where brucellosis has been detected in not more than 0.2 per cent of the holdings during the last two years, and if they are moved from a holding, which has been granted the status of an officially brucellosis-free holding, and if during transportation they do not come in contact with animals, the health status of which is worse;

35.3. the animals are from a holding, which has the status of a brucellosis-free holding in accordance with the requirements referred to in Paragraph 36 of this Regulation.

36. The following animals may be moved from a holding that has the status of a brucellosis-free holding to the holding of the same status:

36.1. animals which are over 12 months of age and which have been serologically tested, using the AT and CFT methods, within 30 days before or after their stationing at the holding, and negative results have been obtained. Animals, which have not been serologically tested before their stationing at the holding, shall be isolated, serologically tested and stationed with other animals at the holding only after they have been serologically tested;

36.2. animals under the age of 30 months, which have been vaccinated against brucellosis and which are serologically tested in accordance with Sub-paragraph 31.4 of this Regulation.

2.3. Measures to be Taken in Order for a Holding to be Granted and Retained the Status of an Officially Brucellosis-free Holding, if the Status of an Officially Brucellosis-free Country has Been Suspended for Latvia

2.3.1. Granting of the Status of an Officially Brucellosis-free Holding

37. If the status of an officially brucellosis-free country has been suspended for Latvia and:

37.1. the status of an officially brucellosis-free holding has been suspended for a holding, the animal owner or holder may apply to the Service for granting of the status of an officially brucellosis-free holding (Annex 2), provided that they have taken the eradication measures of brucellosis referred to in Sub-chapter 3.3 of this Regulation and complied with the requirements referred to in Paragraph 38 of this Regulation;

37.2. the status of an officially brucellosis-free holding has been previously retained for a holding, the animal owner or holder shall implement and ensure the preventive measures referred to in Sub-chapter 2.3.2 of this Regulation. In such case the status of an officially brucellosis-free holding shall be retained for the holding in future as well.

38. The Service shall grant the status of an officially brucellosis-free holding to a holding within one month after receipt of the application (Annex 2), if at the holding:

38.1. animals have not been vaccinated against brucellosis, except female animals, which have been vaccinated three years earlier, before the status of an officially brucellosis-free holding was requested;

38.2. clinical symptoms characteristic to brucellosis have not been observed in animals during last six months;

38.3. animals which are more than 12 months old have been serologically tested in accordance with one of the following testing schemes:

38.3.1. blood serum samples of animals are serologically tested twice with an interval that is not less than three months and not longer than 12 months;

38.3.2. milk samples of animals are serologically tested by using the ELISA method three times a year with an interval of at least three months and blood serum

samples of animals are serologically tested not earlier than six weeks after testing of the third milk sample;

38.4. animals from another holding have been placed in the holding in accordance with the requirements referred to in Paragraph 39 of this Regulation.

39. The animal owner or holder may station animals from a holding, which has been granted the status of an officially brucellosis-free holding, at their holding, if:

39.1. the age of animals exceeds 12 months, they have been serologically tested by using the AT method not later than 30 days before their stationing at the holding and negative results have been obtained, or if animals have been serologically tested by using any other method referred to in Sub-paragraph 12.1 of this Regulation;

39.2. before stationing of the animals, which have not been subject to serological testing, at the holding, they shall be isolated and serologically tested.

2.3.2. Retaining of the Status of an Officially Brucellosis-free Holding

40. The status of an officially brucellosis-free holding shall be retained for a holding, if:

40.1. each year control samples are taken from animals at the holding in accordance with the requirements referred to in Paragraph 34 of this Regulation and the animals are serologically tested;

40.2. animals are stationed at the holding in accordance with Paragraph 41 of this Regulation.

41. The animal owner or holder may station animals at a holding, which has been granted the status of an officially brucellosis-free holding, in one of the following cases:

41.1. if animals are transferred from a holding of the same status and have been serologically tested in accordance with Paragraph 39 of this Regulation;

41.2. serological testing of the animals referred to in Sub-paragraph 41.1 of this Regulation need not be performed, if they are moved from the country or an administrative territory where brucellosis has been detected in not more than 0.2 per cent of the holdings during the last two years and if they are moved from a holding, which has been granted the status of an officially brucellosis-free holding and if during transportation they do not come in contact with animals, the health status of which is worse;

41.3. animals from a holding, which has the status of a brucellosis-free holding, if:

41.3.1. the animals stationed are more than 18 months old and vaccination against brucellosis of these animals has been performed not more than one year before moving;

41.3.2. animals have been serologically tested by using the AT method and negative results have been obtained not later than 30 days before moving, or the animals have been serologically tested by using any other method referred to in Sub-paragraph 12.1 of this Regulation;

41.3.3. female animals are moved from a holding, if two years have passed since granting of the status of a brucellosis-free holding to such holding, and no animals vaccinated against brucellosis have been stationed at the respective holding for two years.

42. If the status of an officially brucellosis-free country has not been granted to Latvia, but in the country:

42.1. brucellosis has been detected in not more than one per cent of bovine holdings, the Service shall determine that milk samples shall be tested for animals who are more than 12 months old, using the ELISA method in all holdings twice a year with an interval of at

least three months, or blood serum samples of an animal shall be serologically tested, using any other of the methods referred to in Sub-paragraph 12.1 of this Regulation once a year;

42.2. at least 99.8 per cent of bovine holdings have the status of an officially brucellosis-free holding for at least four years, the Service shall choose one of the following testing schemes at the holding:

42.2.1. serological testing of blood serum samples for animals older than 12 months shall be performed once every two years;

42.2.2. serological testing of blood serum samples for animals older than 24 months shall be performed once a year.

3. Eradication Measures of Brucellosis

43. The animal owner or holder shall inform a practicing veterinarian or the respective territorial unit of the Service within one day, using any means of communication, regarding an animal in which an abortion has occurred, as well as in case if contracting of brucellosis or being infected with brucellosis is suspected.

44. If the practicing veterinarian is not authorised to take eradication measures of brucellosis, he or she shall, after receipt of the information referred to in Paragraph 43 of this Regulation, inform the respective territorial unit of the Service without delay.

45. The territorial unit of the Service, within one day after receipt of the information referred to in Paragraph 43 or 44 of this Regulation, shall inform, using any means of communication (in writing, electronically or orally):

45.1. the animal owner or holder regarding the official veterinarian or the inspector of the Service, who will take eradication measures of brucellosis;

45.2. regarding the official veterinarian or the inspector of the Service.

46. The official veterinarian or the inspector of the Service, upon commencing the eradication measures of brucellosis, shall, within one day, perform clinical examination of the animal, evaluate the epizootiological situation and check whether the required testing of control samples has been performed.

47. The official veterinarian or the inspector of the Service shall take a sample of clinical or pathological material in accordance with the procedures referred to in Paragraph 46 of this Regulation and send them for laboratory testing to the Institute in order to determine the diagnosis of brucellosis in accordance with the requirements referred to in Paragraph 15 or 16 of this Regulation, taking into account whether the status of an officially brucellosis-free country has been retained or suspended for Latvia.

48. The animal, which is suspected regarding infection or illness with brucellosis, shall be labelled and held separately from other animals at the holding, thus protecting them from becoming infected with brucellosis.

49. The Service has the right to determine that at a holding where fattening bulls are grown, after isolation of the animal suspected of being infected or ill with brucellosis, castrated animals may be stationed, if they are sent to slaughterhouse after fattening.

50. It is prohibited to move animals outside the holding until a diagnosis has been determined or outside the affected area at the time when eradication measures of brucellosis are taken (hereinafter – limitation period), except the movement of animals to the slaughterhouse for immediate slaughtering or except the case referred to in Paragraph 49 of this Regulation.

51. The animal, which is suspected of being infected with brucellosis, may be utilised after slaughtering for the production of animal feed or food, by thermally processing the slaughter products in the manner that destroys the brucellosis agent.

52. The milk, which has been obtained from an animal, which is suspected of being infected with brucellosis, may:

52.1. be fed to the animals at the holding, if the milk has been thermally processed in the manner that destroys the brucellosis agent;

52.2. be delivered to milk processing or milk treatment company in order to perform thermal treatment of the milk in the manner that destroys the brucellosis agent. After appropriate thermal processing, the milk may be used for food processing.

53. In order to collect a sample of pathological material for bacteriological testing, the official veterinarian or the inspector of the Service, if necessary, shall slaughter the animal, if there are suspicions regarding infection of animal with brucellosis. The animal owner or holder shall utilise, process or dispose of the slaughtered animal in accordance with the instructions of the inspector, applying the processing methods and conditions laid down in Regulation No 1069/2009 and Regulation No 142/2011.

54. The relevant territorial unit of the Service, upon receipt of information regarding a confirmed diagnosis of brucellosis, shall, within a working day:

54.1. perform the research of epizootiological situation;

54.2. determine and approve a plan of eradication measures of brucellosis in accordance with Paragraph 56 of this Regulation;

54.3. determine the area affected by brucellosis in accordance with Paragraph 57 of this Regulation.

55. The relevant territorial unit of the Service shall inform the following parties regarding the measures referred to in Paragraph 54 of this Regulation within two working days:

55.1. the relevant local government;

55.2. the official veterinarian;

55.3. the Centre for Disease Prevention and Control.

56. The plan of eradication measures of brucellosis shall lay down the eradication measures of brucellosis at the affected area, indicating the sequence of the measures (which will ensure control of the eradication measures of brucellosis, epidemiological testing and human observation, actions with animals, raw milk, fresh milk, fresh meat, slaughter products and by-products), as well as the term of limitations and other measures.

57. The affected area shall be determined, taking into account:

57.1. the results of the research of epizootiological situation in the particular territory, determining the area of origin of the brucellosis agent, the ways of spreading and the possible infected objects;

57.2. geographical and administrative borders;

57.3. social factors and density of animal population.

58. After performance of the actions referred to in Paragraph 54 of this Regulation, the Service shall:

58.1. within one working day post information on the confirmed case of brucellosis on the website of the Service, indicating:

58.1.1. the date when the diagnosis of brucellosis was confirmed;

58.1.2. the animal species;
58.1.3. the number of animals;
58.1.4. the area affected by brucellosis;
58.1.5. the minimum time of restrictions;

58.2. review the status of the holding within one working day and grant the respective status to the holding in accordance with Paragraph 59 of this Regulation;

58.3. report to the European Commission and other Member States of the European Union on a confirmed diagnosis of brucellosis in accordance with Paragraph 24 of this Regulation.

59. The Service shall:

59.1. grant or suspend one of the following statuses to the holding affected by brucellosis – undefined brucellosis status, defined brucellosis status, the status of a brucellosis-free holding or the status of an officially brucellosis-free holding;

59.2. take a decision:

59.2.1. to slaughter such animals at the affected area, which are infected or which are suspected regarding illness with brucellosis, and to utilise, process or dispose of these animals 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;

59.2.2. to send all animals stationed at the affected area for slaughtering to the slaughterhouse or to laboratory testing, if there are suspicions regarding infection with brucellosis, or if they are susceptible to brucellosis;

59.2.3. on laboratory testing of all other animal species at the affected area that are susceptible to brucellosis.

60. The animal owner or holder shall fulfil the requirement referred to in Sub-paragraph 59.2.1, 59.2.2 or 59.2.3 of this Regulation within 30 days.

61. The Service has the right to determine that after slaughtering of the animals referred to in Sub-paragraph 59.2.1 of this Regulation, the animal owner or holder at the affected area:

61.1. may bring out castrated fattening bulls to the holding of the same status, from which they are sent to a slaughterhouse after fattening;

61.2. may grow or bring in animals, if they are not younger than 12 months and have been serologically tested in accordance with the procedures laid down for the respective status of the holding before growing or bringing them in. If the animals to be brought in have been vaccinated, serological testing shall be performed after they reach the age of 18 months;

61.3. shall ensure serological testing for the animals until the holding conforms to the status of a brucellosis-free holding or the status of an officially brucellosis-free holding.

62. Animals shall be moved from one holding to another according to the following procedures:

62.1. animals from a holding with an undefined brucellosis status may be moved to a holding of the same status or to a holding of a defined brucellosis status, if the requirements referred to in Paragraph 63 of this Regulation are conformed to;

62.2. animals from a holding with a defined brucellosis status may be moved to a holding of the same status or to a holding with an undefined brucellosis status, if the requirements referred to in Paragraph 64 of this Regulation are conformed to;

62.3. animals from a holding with the status of a brucellosis-free holding or the status of an officially brucellosis-free holding may be moved to a holding with undefined or defined brucellosis status;

62.4. animals from a holding with the status of a brucellosis-free holding may be moved to a holding with the status of an officially brucellosis-free holding, and vice versa, if the requirements referred to in Paragraph 35, 39 or 41 of this Regulation are conformed to.

63. Upon moving of the animals from a holding with an undefined brucellosis status to a holding with a defined brucellosis status, the following procedures shall be conformed to:

63.1. the animals, the age of which exceeds 12 months, shall be serologically tested not later than 30 days before movement;

63.2. the moved animals shall be isolated for at least 60 days. The animals, the age of which exceeds 12 months, shall be repeatedly serologically tested and added to a herd if the testing results are negative.

64. Upon moving of the animals from a holding with a defined brucellosis status to a holding of the same status, the following procedures shall be conformed to:

64.1. the animals, the age of which exceeds 12 months, shall be serologically tested not later than 30 days before movement;

64.2. before movement and during movement the animals may not come into contact with other animals the health status of which is worse.

65. After carrying out of the activities referred to in Paragraph 59 of this Regulation, the animal owner or holder, under the supervision of the inspector of the Service or an official veterinarian, shall ensure the destruction of the brucellosis agent:

65.1. by treating or processing the manure, which has been in contact with serologically positive animals or sources of brucellosis agents (placenta, aborted foetus, embryo, stillborn or dead calf), using the processing methods and conforming to the conditions that are laid down in Regulation No 1069/2009 and Regulation No 142/2011;

65.2. by cleaning, washing and disinfecting the holding, equipment, inventory of the holding and vehicles, which have been used for transportation of the respective animals, with the agent that destroys the brucellosis agent.

66. The inspector of the Service shall collect disinfection quality control samples after disinfection and send them to the Institute.

67. Manure and slurry, which have been collected from serologically negative animals, after its impregnation with disinfectant, shall be placed for bio-thermal treatment in sites where it does not cause the risk of infection, and shall be stored for not less than three weeks, in order to allow it reach the appropriate temperature and kill the brucellosis agent.

68. Grazing grounds in the territory of a holding affected by brucellosis shall be re-utilised not earlier than 60 days after slaughtering of serologically positive animals. This condition needs not be observed, if the grazing grounds are intended for castrated fattening bulls and these animals are sent to a slaughterhouse or another holding of the herd, from which they are sent to the slaughterhouse after fattening.

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

70. The respective local government shall inform the inhabitants of its administrative territory regarding the measures taken in order to restrict brucellosis.

71. The Centre for Disease Prevention and Control shall ensure epidemiological testing of such persons at the affected area who have been in contact with the infected animal or its

corpse, as well as organise laboratory testing and medical follow-up of such persons in accordance with the regulatory enactment regarding the procedures for the determination of exposed persons, initial medical examination, laboratory examination and medical observation.

72. The official veterinarian shall inform the respective territorial unit of the Service regarding implementation of the plan of eradication measures of brucellosis in writing within two working days thereafter, but the Centre for Disease Prevention and Control shall inform the Service regarding the results of epidemiological testing and anti-epidemic measures.

73. The territorial unit of the Service, after evaluation of the information referred to in Paragraph 72 of this Regulation, shall decide on retaining or revocation of the status of the affected area.

3.1. Measures to be Taken, if the Status of an Officially Brucellosis-free Country is Retained for Latvia

74. Upon receipt of a positive analysis result, which was carried out by using the ELISA, AT or RBT and CFT method, the Service shall suspend the status of a brucellosis-free holding or an officially brucellosis-free holding for a holding until the diagnosis is established, and shall renew or fully revoke the corresponding status of the holding after establishment of the diagnosis.

75. If it is suspected that the animal is infected or ill with brucellosis, or the diagnosis of brucellosis is confirmed, the Service shall take a decision on action with the animal in accordance with the requirements referred to in Sub-paragraph 59.2 of this Regulation.

76. The Service shall ensure taking of blood samples and carrying out of serological testing by using the RBT and CFT methods to detect the specific antibodies against the brucellosis agent in all susceptible animals at the holdings that are epizootiologically linked with the affected area.

77. The Service shall report to the European Commission and other Member States of the European Union on the measures taken at the affected area.

3.2. Measures to be Taken in Order to Suspend or Renew the Status of a Brucellosis-free Holding for a Holding, if the Status of an Officially Brucellosis-free Country has Been Suspended for Latvia

78. Upon receipt of a positive analysis result, which has been carried out by using the ELISA, AT or RBT and CFT method, or the information referred to in Paragraph 43 of this Regulation, proving that clinical symptoms characteristic to brucellosis are observed in an animal over 30 months of age and it has been isolated or slaughtered, the Service shall suspend the status of a brucellosis-free holding for the holding until the diagnosis is established and shall renew or fully revoke the status of the holding after establishment of the diagnosis.

79. The animal, from which serologically positive results have been obtained (hereinafter – serologically positive animal) and which has not been vaccinated, and is isolated, may be stationed at the holding with other animals, and the status of a brucellosis-free holding may be renewed for such holding, if the animal has been repeatedly serologically tested, selecting one of the following testing schemes:

79.1. the AT and CFT methods, obtaining negative result;

79.2. any other of the methods referred to in Sub-paragraph 12.1 of this Regulation, which is not referred to in Sub-paragraph 79.1 of this Regulation.

80. If the serologically positive animal has been slaughtered during eradication of brucellosis and repeated sampling is not possible, the status of a brucellosis-free holding shall be renewed for a holding, if all animals over 12 months of age have been serologically tested twice by using the AT method and negative results have been obtained. The first serological testing shall be performed 30 days after isolation of serologically positive animals and the second testing – 60 days after the first testing by using the AT method.

81. If the diagnosis of brucellosis is confirmed, the Service shall fully revoke the status of a brucellosis-free holding and it may be renewed after eradication measures of brucellosis have been completed, if one of the following conditions has been conformed to:

81.1. all animals of the holding have been slaughtered;

81.2. all non-vaccinated animals at the holding, which are older than 12 months, have been serologically tested twice. Serological testing shall be performed for the first time 30 days after isolation of the serologically positive animals and for the second time 60 days after performing the first serological testing, furthermore, pregnant animals are subject to serological testing not earlier than 21 days after parturition of the last pregnant animal.

82. The animals, which are younger than 30 months and have been vaccinated against brucellosis with Strain 19 live vaccine, shall be serologically tested, using:

82.1. the AT method, and the result thereof shall be considered to be negative, if 30 to 80 International Units of specific antibodies against the brucellosis agent are detected per millilitre of the animal blood serum sample;

82.2. the CFT method, and the result thereof shall be considered to be negative, if less than 30 international CFT units against the brucellosis agent per one millilitre of the blood serum sample taken from the tested female animal are detected and if vaccination against brucellosis in these animals has been performed within 12 months before testing, or less than 20 international CFT units against the brucellosis agent per millilitre, if vaccination against brucellosis in the animals has been performed more than one year before testing.

3.3. Measures to be Taken in Order to Suspend or Renew the Status of an Officially Brucellosis-free Holding for a Holding, if the Status of an Officially Brucellosis-free Country has been Suspended for Latvia

83. Upon receipt of a positive analysis result, which has been carried out by using the ELISA, AT or RBT and CFT method, or the information regarding an animal referred to in Paragraph 43 of this Regulation, in which clinical symptoms characteristic to brucellosis are observed, the Service shall suspend the status of an officially brucellosis-free holding for the holding until the diagnosis is established and shall renew or fully revoke the status of the holding after establishment of the diagnosis.

84. A serologically positive animal, which has not been vaccinated, shall be isolated and may be stationed at the holding together with other animals and the status of an officially brucellosis-free holding may be renewed for the holding, if the animal has been repeatedly serologically tested in accordance with Paragraph 79 of this Regulation.

85. If the serologically positive animal has been slaughtered during eradication of brucellosis and repeated sampling is impossible, the status of an officially brucellosis-free holding shall

be renewed for the holding, if all animals have been tested in accordance with Paragraph 80 of this Regulation.

86. If the diagnosis of brucellosis is confirmed, the Service shall fully revoke the status of an officially brucellosis-free holding and it may be renewed after eradication measures of brucellosis are completed, if the conditions referred to in Paragraph 81 of this Regulation are conformed to.

4. Final Provisions

87. In order to perform the control sample tests required in 2013, the Service shall submit information regarding the number of the required control samples and the selection criteria thereof to the Data Centre until 1 February 2013.

88. The Data Centre shall prepare information for animal owners or holders and the Service on the holdings registered in the animal register, in which tests of control samples of the animals are required in 2013, and post it on its website until 31 March 2013.

89. This Regulation shall come into force on 1 January 2013.

Informative Reference to the European Union Directives

This Regulation contains legal norms arising from:

1) Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals and swine; and

2) Council Directive 77/391/EEC of 17 May 1977, introducing Community measures for the eradication of brucellosis, tuberculosis and leucosis in cattle;

3) Council Directive 78/52/EEC of 13 December 1977 establishing the Community criteria for national plans for the accelerated eradication of brucellosis, tuberculosis and enzootic leukosis in cattle.

Prime Minister V. Dombrovskis

Minister for Agriculture L. Straujuma

Annex 1
Cabinet Regulation No. 881
18 December 2012

Description of the Methods of Laboratory Diagnostics of Bovine Brucellosis

1. Identification of the Disease Agent

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 suspicions regarding illness with brucellosis, especially if supported by serological testing methods. Polymerase chain reaction (hereinafter – PCR) may be used as an additional method for the detection of bovine brucellosis agents.

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 tissue, 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 oxydative metabolism test in accordance with cultivation, biochemical and serological criteria. PCR 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 of which is available on the website http://www.oie.int/en/international-standard-setting/terrestrial-manual/access-online/.

2. Immunological Methods

2.1. Standards

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), agglutination test (hereinafter – AT) and complement fixation test (hereinafter – CFT).

6. The standard reference serum for the RBT, AT and CFT is OIE international reference standard serum (hereinafter – OIEISS), formerly named WHO second international anti-*Brucella abortus* Serum (hereinafter – ISAbS).

7. The standard reference serums for immunoenzymatic analysis (hereinafter – ELISA) are:
   7.1. the OIEISS;
   7.2. the weak positive OIE ELISA standard serum (hereinafter – OIEELISA$_{WP}$SS);
   7.3. the strong positive OIE ELISA standard serum (hereinafter – OIEELISA$_{SP}$SS);
   7.4. the negative OIE ELISA standard serum (hereinafter – OIEELISA$_{N}$SS).

8. The standards referred to in Paragraph 7 of this Annex are available at the Community Reference Laboratory for Brucellosis or Weybridge Veterinary Laboratories Agency (VLA) in the United Kingdom.

9. The OIEISS, the $OIEELISA_{WP}SS$, the $OIEELISA_{SP}SS$ and the $OIEELISA_{N}SS$ are international primary standards, from which secondary reference national standards must be established in each Member State for all methods referred to in Paragraph 5 of this Annex (hereinafter – working standards).

2.2. Immunoenzymatic Analysis or Other Laboratory Methods in Order to Determine Bovine Brucellosis in Blood Serum or Milk

2.2.1. Materials and Reagents

10. Testing shall be performed by an accredited laboratory, using methods which have been approved by the OIE as methods confirming bovine brucellosis and which have been approved by an accredited laboratory.

2.2.2. Standardisation of the Laboratory Method

11. Standardisation of the laboratory method for individual blood serum samples:

11.1. 1/150 pre-dilution of the OIEISS (the dilutions referred to in this Annex, in order to create the reagents, shall be expressed as, for instance, 1/150 (which means 1 unit in 150-fold dilution)) or 1/2 pre-dilution of the $OIEELISA_{WP}SS$, or 1/16 pre-dilution of the $OIEELISA_{SP}SS$, which is made up in a negative serum (or in a negative pool of sera), which gives a positive reaction;

11.2. 1/600 pre-dilution of the OIEISS or 1/8 pre-dilution of the $OIEELISA_{WP}SS$, or 1/64 pre-dilution of the $OIEELISA_{SP}SS$, which is made up in a negative serum (or in a negative pool of sera), which gives a negative reaction;

11.3. the $OIEELISA_{N}SS$ always gives a negative reaction.

12. Standardisation of the laboratory method of pooled blood serum samples:

12.1. 1/150 pre-dilution of the OIEISS or 1/2 pre-dilution of the $OIEELISA_{WP}SS$, or 1/16 pre-dilution of the $OIEELISA_{SP}SS$, which is made up in a negative serum (or in a negative pool of sera) and again diluted in negative sera by the number of samples making up the pool and giving a positive reaction;

12.2. the $OIEELISA_{N}SS$ always gives a negative reaction;

12.3. the laboratory method is adequate to detect the specific antibodies against the bovine brucellosis agent in the blood serum of one animal, which has been collected and pooled into a blood sample of several animals.

13. Standardisation of the laboratory method of pooled milk or whey samples:

13.1. 1/1000 pre-dilution of the OIEISS or 1/16 pre-dilution of the $OIEELISA_{WP}SS$, or 1/125 pre-dilution of the $OIEELISA_{SP}SS$, which is made up in a negative serum (or a negative pool of sera) and again diluted 1/10 in negative milk and gives a negative reaction;

13.2. the $OIEELISA_{N}SS$, diluted 1/10 in negative milk, always gives a negative reaction;

13.3. the laboratory method is adequate to detect the specific antibodies against the bovine brucellosis agent in the milk or whey sample of one animal, which has been collected and pooled into a milk or whey sample of several animals.

2.2.3. Conditions for the Use of Immunoenzymatic Analysis for Diagnosis of Bovine Brucellosis

14. Using the calibrating conditions for ELISA blood serum samples referred to in Paragraphs 11 and 12 of this Annex, the diagnostic sensitivity of ELISA shall be equal or greater than the diagnostic sensitivity of RBT or CFT, taking into account the epidemiological situation under which it is employed.

15. Using the calibrating conditions for ELISA pooled milk samples referred to in Paragraph 13 of this Annex, the diagnostic sensitivity of ELISA shall be equal or greater than the diagnostic sensitivity of milk ring test, taking into account not only the epidemiological situation, but also the average and expected extreme husbandry system.

16. Where ELISA is used for certification purposes or in order to grant and retain the status of a holding, pooling of samples must be carried out in such a way that ELISA results can be undoubtedly related to the individual animal included in the pool. The diagnosis of bovine brucellosis is confirmed for each animal individually, using serological testing in order to detect the specific antibodies against the bovine brucellosis agent in blood serum sample.

17. The ELISA may be used on a sample of milk taken from a holding with at least 30% of dairy cows in milk. If this method is used, measures must be taken to ensure that the samples taken for examination can be undoubtedly related to the individual animals from which the milk is derived. The diagnosis of bovine brucellosis is confirmed for each animal individually, using serological testing in order to detect the specific antibodies against the bovine brucellosis agent in a blood serum sample.

2.3. Complement Fixation Test

18. 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 label of the package. The antigen must be stored at the temperature of 4 °C and not frozen.

19. Blood serum of bovine animals must be inactivated for 30 to 50 minutes at the temperature of 56–60 °C.

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

21. Each time, when the CFT is carried out, the following controls shall be made:
- 21.1. control of the anti-complementary effect of the blood serum;
- 21.2. control of the antigen;
- 21.3. control of sensitised red blood cells;
- 21.4. control of the complement;
- 21.5. control using a positive serum of sensitivity at the start of the reaction;
- 21.6. control of the specificity of the reaction using a negative serum.

2.3.1. Calculation of Results

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

23. If the OIEISS is tested in a given method, the result is given as a titre (i.e., the highest 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:

$$ICFTU_{TESTSERUM} = F \times T_{TESTSERUM}$$

24. Interpretation of CFT results – testing result is considered to be positive, if 20 or more ICFTU to the bovine brucellosis agent are detected per millilitre of a blood serum sample.

2.4. Rose Bengal Test

25. The antigen represents a bacterial suspension in buffered solution with a pH of 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.

26. 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 with a blood serum dilution of 1:45 and a negative reaction with a dilution of 1 : 55.

27. Conditions for the use of RBT:

27.1. blood serum (20–30 µl) is mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone 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;

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

28. Interpretation of RBT results – 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.

2.5. Agglutination Test

29. The antigen represents a bacterial suspension in phenol-saline (NaCl 0,85 % (m/v) and phenol at 0,5 % phenol (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, EDTA may be added to the antigen suspension to 5 mM final test dilution in order to reduce the risk of false positives.
pH of 7,2 must be readjusted in the antigen suspension.

30. The OIEISS contains 1000 international units of agglutination.

31. 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 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.

32. The AT is 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 must be 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 is made in the median tube (or well for the microplate method).

33. Interpretation of AR results – the degree of agglutination in a blood serum must be expressed in international units per one millilitre (hereinafter – IU/ml). Test result shall be considered to be positive, if 30 or more IU/ml against the bovine brucellosis agent are detected in the blood serum sample.

3. Tasks and Duties of the State Reference Laboratory

34. State reference laboratories shall be responsible for:

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

34.2. determining the maximum number of samples pooled, using ELISA reagent kits;

34.3. calibration of standard secondary benchmarks for the national standard serum ("working standards") in relation to the primary international standard serum referred to in Sub-chapter 2.1 of this Annex;

34.4. quality checks of all antigen and ELISA reagent kit batches used in the Member State;

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

Minister for Agriculture L. Straujuma

Annex 2
Cabinet Regulation 881
18 December 2012

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

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

_____

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

_____
_____
_____

3. Contact details:
3.1. telephone or mobile telephone 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 Agricultural Data Centre

L =	V							

6. Holding registration number in the register of Agricultural Data Centre*

L =	V							

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

8. To grant the following status to the holding (mark as appropriate):
8.1. [ ] status of a brucellosis-free holding
8.2. [ ] status of an officially brucellosis-free holding

9. At the holding:
9.1. clinical symptoms of brucellosis (have/have not) _____ been observed in animals during the last years;
9.2. the following animals are kept (indicate the number by animal group – bulls for breeding, cows, calves)

_____
_____
_____
_____

9.3. animals have been serologically tested during the last 12 months (testing review No.)**

(date***)

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

Notes.
1. * Also indicate the internal number 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 regulatory enactment regarding the drawing up of electronic documents.

Minister for Agriculture L. Straujuma