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To:	Mr Jeppe TRANHOLM-MIKKELSEN, Secretary-General of the Council of the European Union
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Subject:	ANNEXES to the COMMISSION DELEGATED REGULATION (EU) .../... supplementing Regulation (EU) 2016/429 of the European Parliament and the Council laying down rules for surveillance, eradication programmes and disease freedom for certain listed and emerging diseases

Delegations will find attached document C(2019) 4056 final ANNEXES 1 to 6.

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ANNEXES 1 to 6

ANNEXES

to the

COMMISSION DELEGATED REGULATION (EU) .../...

supplementing Regulation (EU) 2016/429 of the European Parliament and the Council laying down rules for surveillance, eradication programmes and disease freedom for certain listed and emerging diseases

ANNEX I

SPECIFIC CASE DEFINITION OF DISEASE OF TERRESTRIAL ANIMALS

SECTION 1
HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI)

1. An animal or a group of animals must be considered, by the competent authority, as a suspected case of HPAI when it meets the criteria laid down in Article 9(1).

2. An animal or a group of animals must be considered, by the competent authority, as a confirmed case of HPAI when:

   (a) the disease agent responsible for HPAI, excluding vaccine strains, has been isolated in a sample from an animal or from a group of animals;

   (b) nucleic acid specific to the disease agent for HPAI, that is not a consequence of vaccination, has been identified in a sample from an animal or from a group of animals; or

   (c) positive result to an indirect diagnostic method, that is not a consequence of vaccination, has been obtained in a sample from a kept animal or from a group of kept animals showing clinical signs consistent with the disease or epidemiologically linked to a suspected or confirmed case.

3. For the purposes of this case definition, the disease agent responsible for HPAI must be either

   (a) an influenza A virus of H5 and H7 subtypes or any influenza A virus with an intravenous pathogenicity index (IVPI) greater than 1.2; or

   (b) an influenza A virus of H5 and H7 subtypes with a sequence of multiple basic amino acids present at the cleavage site of the haemagglutinin molecule (HA0) that is similar to that observed for other HPAI isolates.

SECTION 2
INFECTION WITH LOW PATHOGENIC AVIAN INFLUENZA VIRUSES (LPAIV)

1. An animal or a group of animals must be considered, by the competent authority, as a suspected case of infection with LPAIV when it meets the criteria laid down in Article 9(1).

2. An animal or a group of animals must be considered, by the competent authority, as a confirmed case of infection with LPAIV when:

   (a) the disease agent responsible for infection with LPAIV, excluding vaccine strains, has been isolated in a sample from an animal or from a group of animals;

   (b) nucleic acid specific to the disease agent for infection with LPAIV, that is not a consequence of vaccination, has been identified in a sample from an animal or from a group of animals; or

   (c) positive result to an indirect diagnostic method, that is not a consequence of vaccination, has been obtained in a sample from a kept animal or from a group of kept animals showing clinical signs consistent with the disease or epidemiologically linked to a suspected or confirmed case.

3. For the purposes of this case definition, the disease agent of infection with LPAIV must be any influenza A virus of H5 and H7 subtypes that are not HPAI viruses.

SECTION 3
INFECTION WITH NEWCASTLE DISEASE VIRUS (NDV)

1. An animal or a group of animals must be considered, by the competent authority, as a suspected case of infection with NDV when it meets the criteria laid down in Article 9(1).

2. An animal or a group of animals must be considered, by the competent authority, as a confirmed case of infection with NDV when:

   (a) the disease agent responsible for infection with NDV, excluding vaccine strains, has been isolated in a sample from an animal or from a group of animals;

   (b) nucleic acid specific to the disease agent for infection with NDV, that is not a consequence of vaccination, has been identified in a sample from an animal or from a group of animals; or

   (c) positive result to an indirect diagnostic method, that is not a consequence of vaccination, has been obtained in a sample from a kept animal or from a group of kept animals showing clinical signs consistent with the disease or epidemiologically linked to a suspected or confirmed case.

3. For the purposes of this case definition, the disease agent responsible for infection with NDV must be any avian paramyxovirus type 1 (APMV-1) (avian *Avulavirus* type 1) that either:

   (a) has an intracerebral pathogenicity index (ICPI) of 0.7 or greater; or

   (b) presents multiple basic amino acids at the C-terminus of the F2 protein and phenylalanine at residue 117, which is the N-terminus of the F1 protein. The term ‘multiple basic amino acids’ refers to at least three arginine or lysine residues between residues 113 and 116. Failure to demonstrate the characteristic pattern of amino acid residues as described above would require characterisation of the isolated virus by an ICPI test. In this definition, amino acid residues are numbered from the N-terminus of the amino acid sequence deduced from the nucleotide sequence of the F0 gene (113–116 corresponds to residues –4 to –1 from the cleavage site).

ANNEX II

UNION SURVEILLANCE PROGRAMME

Part I
Avian influenza surveillance in poultry and wild birds

SECTION 1
GENERAL APPROACH AND REQUIREMENTS

1. TERRITORIAL SCOPE

Surveillance in poultry and wild birds must be implemented in all Member States.

2. PERIOD OF APPLICATION

Until revoked.

3. GENERAL APPROACH

The surveillance system must address the objectives provided for in Section 2 and must be built on a comprehensive approach including different components of surveillance activities complementing each other in poultry and wild bird populations:

- Early detection systems as provided for in Sections 3 and 4;
- Risk-based surveillance as provided for in Sections 5 and 6.

SECTION 2
OBJECTIVES FOR SURVEILLANCE IN POULTRY AND WILD BIRDS

1. Early detection of highly pathogenic avian influenza (HPAI) in poultry.
2. Early detection of HPAI in wild birds providing for:
   (a) an early warning for possible HPAI introduction into poultry, in particular when viruses enter the Union through migratory movements of wild birds;
   (b) information for the assessment of risks for virus spread following findings of HPAI in wild birds.
3. Detection of HPAI in poultry species which generally do not show significant clinical signs.
4. Detection of circulating low pathogenic avian influenza viruses (LPAIV) that may easily spread between poultry flocks in particular in areas with a high density of poultry establishments in view of their potential to mutate to HPAI in order to:
   (a) identify clusters of infection with LPAIV; and
   (b) monitor the risk of spread of LPAIV by movements of poultry and by fomites in certain production systems at risk.
5. Contribution to increased knowledge on HPAI and LPAIV posing a potential zoonotic risk.

SECTION 3
EARLY DETECTION OF HPAI IN POULTRY

1. The early detection systems for of HPAI in poultry must be part of the general surveillance requirements as provided for in point (a) of Article 3(1) and must be implemented throughout the poultry sector.

2. The surveillance referred to in point 1 must at least include the early detection and investigation in establishments located in an area identified as being at heightened risk for HPAI introduction and spread, of:

   (a) any change in normal production and health parameters such as mortality rate, feed and water intake and egg production; and

   (b) any clinical sign or post-mortem lesion suggesting HPAI.

3. Regular testing of samples collected from dead and sick poultry in establishments located in an area identified as being at heightened risk for HPAI introduction and spread may also be relevant when an increased risk has been identified at national, EU or regional level due to outbreaks of HPAI in poultry and/or wild birds.

SECTION 4
EARLY DETECTION OF HPAI IN WILD BIRDS

1. The early detection of HPAI in wild birds must be based on sampling and testing of birds that have been:

   (a) found dead;

   (b) found injured or sick;

   (c) hunted with clinical signs.

   This surveillance may need to be increased, when HPAI has been detected in wild birds, by monitoring systems using organised patrols for detecting and collecting dead and sick birds.

2. The design of this surveillance must be risk-based, taking into account at least relevant information on ornithology, virology, epidemiology and environmental matters.

3. The surveillance must apply to birds from targeted wild bird species, as provided for in Section 8. However, all suspected episodes of mortality in wild birds must be investigated to exclude HPAI.

   In addition to targeted wild bird species, additional wild bird species may also be included when their specific epidemiological relevance on the Member State's territory has been assessed.

4. In addition, the surveillance may include, at priority locations and key sites in particular those where birds of targeted wild birds species are entering the Union during their migratory movements, at least from North-East and Eastern routes, the sampling and testing of:

   (a) birds trapped;

   (b) hunted healthy birds;

(c) sentinel birds.

5. Additional sources of information obtained from investigations of wild birds in the context of HPAI outbreaks in kept birds must be included in the results of the surveillance of HPAI in wild birds.

SECTION 5

RISK-BASED COMPLEMENTARY SURVEILLANCE FOR HPAI IN POULTRY SPECIES WHICH GENERALLY DO NOT SHOW SIGNIFICANT CLINICAL SIGNS

1. The risk-based surveillance for infection with HPAI in poultry establishments keeping ducks, geese, poultry belonging to the species of *Anseriformes* for supplies of game or quails to be released into the wild must take into account at least the following risk factors:

   (a) the historical and current epidemiological situation of the disease and its evolution over time in poultry and wild birds;

   (b) the proximity of establishments to water bodies and other places where migratory birds, in particular water birds, may gather in higher numbers or have their stop-over places during their movements into and through the Union;

   (c) the period of increased movements of migratory wild birds of targeted species into and through the Union;

   (d) the structure of poultry farming including the broader sector involved in the different production systems;

   (e) the geographical location of the establishments in an area with a high density of poultry;

   (f) the biosecurity practices on the establishments;

   (g) the type and frequency of movements of poultry, products and vehicles transporting poultry and trade patterns; and

   (h) the risk assessments and scientific advice in relation to the relevance of the spread of HPAI by wild birds.

2. Based on scientific justifications, additional risk factors than those listed in points (a) to (h) of point 1 may be included and factors that are not relevant for the specific situation of the Member State may be omitted.

SECTION 6

RISK-BASED SURVEILLANCE IN ORDER TO IDENTIFY CLUSTERS OF ESTABLISHMENTS INFECTED WITH LPAIV AND WITH CONTINUOUS SPREAD OF LPAIV

1. The risk-based surveillance for the detection of circulating low pathogenic avian influenza viruses (LPAIV) that may easily spread between poultry flocks in particular in areas with a high density of poultry establishments, as referred to in point 4 of Section 2, must apply to poultry establishments for which the competent authority has assessed that clusters of infection with LPAIV have repeatedly occurred in the past or are deemed more likely to occur.

2. Such clusters are characterised by infection with LPAIV of groups of establishments related in time and geographical proximity.

3. The assessment for the selection of establishments for targeted surveillance must take into account the risk for lateral transmission of the virus due to the structure and complexity of the production system and functional connections between establishments, in particular when operating in areas with a high density of establishments.

4. In addition to the selection criteria for targeted surveillance of establishments referred to in point 3, the following risk factors must be taken into account at the establishment level:

   (a) the kept species;

   (b) the cycle and duration of production;

   (c) presence of several poultry species;

   (d) presence of multi-age poultry flocks;

   (e) presence of long-lived poultry;

   (f) practice of all-in all-out principle;

   (g) length of waiting period between batches; and

   (h) biosecurity practices and housing conditions.

SECTION 7
TARGETED POULTRY POPULATIONS

1. Early detection systems for infection with HPAI referred to in Section 3 must apply to all poultry populations.

2. Complementary surveillance for infection with HPAI referred to in Section 5 in poultry species that do generally not display significant signs when infected with HPAI must apply to:

   (a) breeding ducks

   (b) breeding geese;

   (c) fattening ducks;

   (d) fattening geese;

   (e) quails;

   (f) poultry of species belonging to *Anseriformes* for supplies of game to be released into the wild.

3. In addition to the species and categories listed under point 2 the targeting of sampling and testing for infection with LPAIV referred to in Section 6 may apply to the following poultry species and production categories:

   (a) laying hens including those kept in free-range;

   (b) breeding turkeys;

   (c) fattening turkeys;

(d) the poultry of species belonging to *Galliformes* for supplies of game to be released into the wild.

SECTION 8
TARGETED WILD BIRD POPULATIONS

Targeted wild birds species, in particular migratory water birds have shown to be at higher risk of becoming infected with, and transmitting HPAI.

The list of 'wild bird targeted species' compiled and updated in the light of the most recent knowledge is available on the website of the EURL.

SECTION 9
SAMPLING AND LABORATORY TESTING METHODS

1. The number of poultry establishments to be sampled and the number of poultry to be tested per establishment and, as appropriate, by epidemiological unit (e.g. poultry flock, shed, etc.) on the concerned establishment must be based on a statistically valid sampling method. This method may be that used for representative sampling; i.e. an estimated prevalence to be detected according to a pre-defined level of confidence determined by the competent authority.

2. Frequency and period for testing:

   (a) the frequency for sampling and testing of poultry establishments must be determined based on the outcome of a risk assessment by the competent authority;

   (b) the time period for sampling must coincide with seasonal production for each production category, but must not compromise the risk-based surveillance approach;

   (c) when relevant, the time period for sampling must take into account the period of heightened risk as referred to in point 3 of Section 3. Samples must be subjected to laboratory testing by virological methods, when taken for:

      (i) early detection of HPAI in poultry referred to in Section 3;

      (ii) early detection of HPAI in wild birds referred to in Section 4;

      (iii) complementary surveillance for HPAI in poultry species which generally do not show significant clinical signs of HPAI referred to in Section 5;

      (iv) follow-up of sero-positive findings referred to in point 4(b).

   For virological testing the prevalence and time window for detection of active infection must be taken into account.

4. Samples must be subjected to laboratory testing by serological methods, when taken for:

   (a) complementary surveillance for HPAI in poultry species which generally do not show significant clinical signs of HPAI referred to in Section 5 supplementing virological testing, as appropriate;

   (b) detection of clusters of LPAIV infected establishments referred to in Section 6. When for technical reasons or other duly justified reasons sampling for serology is not appropriate, virological testing must be performed.

ANNEX III

DIAGNOSTIC METHODS FOR THE GRANTING AND MAINTENANCE OF DISEASE-FREE STATUS FOR CERTAIN DISEASES OF TERRESTRIAL ANIMALS

SECTION 1
INFECTION WITH *BRUCELLA ABORTUS, B. MELITENSIS* AND *B. SUIS*

1. Serological tests
   (a) tests for blood samples
      (i) buffered *Brucella* antigen tests;
      (ii) complement fixation test (CFT)
      (iii) indirect enzyme-linked immunosorbent assay (I-ELISA)
      (iv) fluorescence polarisation assay (FPA)
      (v) competitive enzyme-linked immunosorbent assay (C-ELISA)
   (b) tests for milk samples
      (i) ring test (MRT)
      (ii) I-ELISA
2. Brucellin skin test (BST)

   For the testing as referred to in section 1 and 2 of Chapter 1 of Part I of Annex IV, Brucellin skin test (BST) shall only be used in ovine and caprine animals.

SECTION 2
INFECTION WITH MYCOBACTERIUM TUBERCULOSIS COMPLEX

1. Tuberculin skin tests
   (a) the single intradermal tuberculin test (SITT)
   (b) the comparative intradermal tuberculin test (CITT)
2. Gamma-interferon assay

SECTION 3
ENZOOTIC BOVINE LEUKOSIS

1. Serological tests
   (a) tests for blood samples
      (i) agar gel immuno-diffusion test (AGID)
      (ii) blocking enzyme-linked immunosorbent assay (B-ELISA)
      (iii) I-ELISA
   (b) tests for milk samples
      (i) I-ELISA

SECTION 4
INFECTIOUS BOVINE RHINOTRACHEITIS/INFECTIOUS PUSTULAR VULVOVAGINITIS (IBR/IPV)

	Methods:	**Matrix:**
non-vaccinated bovine animals	BoHV-1 I-ELISA[a]	individual serum samples[d]
		milk samples
	gB B-ELISA[b]	individual serum samples[d]
		individual meat juice samples
DIVA vaccinated bovine animals with a gE-deleted vaccine	gE B-ELISA[c]	individual serum samples
		individual meat juice samples

[a] I-ELISA for the detection of antibodies against BoHV-1 whole virus. Pools of up to 50 milk samples (individual or bulk milk) may be used in tests for granting and up to 100 milk samples (individual or bulk milk) may be used in tests for the maintenance of the status free from IBR/IPV.

[b] B-ELISA for the detection of antibodies against BoHV-1-gB protein. When referred to tests for the detection of antibodies against whole BoHV-1 in Part IV of Annex IV this method may also be used.

[c] B-ELISA for the detection of antibodies against BoHV-1-gE protein. Individual milk samples may be used when testing to proof the maintenance of the status free from IBR/IPV. The samples may be pooled whereat the number of samples per pool may be chosen based on documented evidence that the test is under all circumstances of day to day laboratory work sensitive enough to detect one single positive sample in the pool.

[d] When testing is carried out to proof the maintenance of the status free from IBR/IPV individually collected samples may be pooled. The number of samples per pool may be modulated based on documented evidence that the test system is under all circumstances of day to day laboratory work sensitive enough to detect one weak positive sample in the pool of the modulated size.

SECTION 5
INFECTION WITH AUJESZKY'S DISEASE VIRUS (ADV)

	Methods:	**Matrix:**
non-vaccinated porcine animals	ADV ELISA[a]	individual or up to 5 pooled serum (or plasma) samples
		individual or up to 5 pooled filter paper samples
		individual meat juice samples
DIVA vaccinated porcine animals with a gE-deleted vaccine	gE ELISA[b]	individual serum samples

[a] ELISA for the detection of antibodies against whole ADV, ADV-gB protein or ADV-gD protein. For batch control of ADV-gB kits and ADV-gD kits or whole ADV kits, Community reference serum ADV 1, or sub-standards, must be scored positive at the dilution of 1:2. When referred to tests for the detection of whole ADV in Part V of Annex IV either of these tests may be used.

[b] ELISA for the detection of antibodies against ADV-gE protein. For batch control, Community reference serum ADV 1, or sub-standards, must be scored positive at the dilution 1:8.

SECTION 6
BOVINE VIRAL DIARRHOEA (BVD)

1. Direct methods:
   (a) Real-time reverse transcription PCR
   (b) BVDV antigen detection ELISA
2. Serological tests:
   (a) I-ELISA
   (b) B-ELISA

ANNEX IV

DISEASE-SPECIFIC REQUIREMENTS FOR THE GRANTING, MAINTENANCE, SUSPENSION AND WITHDRAWAL OF THE DISEASE-FREE STATUS AT THE LEVEL OF ESTABLISHMENTS AND DISEASE-SPECIFIC REQUIREMENTS FOR THE GRANTING AND MAINTENANCE OF THE DISEASE-FREE STATUS AT THE LEVEL OF MEMBER STATES OR ZONES

Part I
Infection with *Brucella abortus, B. melitensis* and *B. suis*

Chapter 1
Establishment free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination

SECTION 1
GRANTING OF THE STATUS

1. The status free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination may only be granted to an establishment keeping bovine, ovine or caprine animals if:

   (a) during the past 12 months there has been no confirmed case of infection with *Brucella abortus, B. melitensis* and *B. suis* in bovine, ovine or caprine animals kept in the establishment;

   (b) during the past 3 years none of the bovine, ovine or caprine animals in the establishment has been vaccinated against infection with *Brucella abortus*, *B. melitensis* and *B. suis*;

   (c) the entire bovine animals over 12 months of age and the entire ovine or caprine animals over 6 months of age present in the establishment at the time of sampling have tested negative to serological test, on two occasions as follows:

      (i) the first test must be carried out on samples taken not earlier than 3 months after the removal of the last confirmed case and of the last animal that tested positive in an immunological test;

      (ii) the second test must be carried out on samples taken not earlier than 6 months and not later than 12 months following the date of sampling referred to in point (i);

   (d) animals showing clinical signs consistent with infection with *Brucella abortus*, *B. melitensis* and *B. suis*, such as abortions, have been subjected to investigations with negative results;

   (e) since the beginning of the sampling referred to in point (c)(i) all bovine, ovine or caprine animals introduced into the establishment originate from establishments free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination, or free with vaccination and have not been vaccinated against infection with *Brucella abortus*, *B. melitensis* and *B. suis* during the past 3 years, and

(i) originate from a Member State or a zone free from infection with *Brucella abortus, B. melitensis* and *B. suis* for the relevant animal population;

(ii) are entire bovine animals over 12 months of age or entire ovine or caprine animals over 6 months of age and must have tested negative in a serological test carried out on a sample taken:

– during the 30 days prior to their introduction into the establishment; or

– during the 30 days following their introduction provided they have been kept isolated during this period; or

(iii) are post-parturient females kept in isolation since their introduction into the establishment until they have tested negative in a serological test carried out on a sample taken not earlier than 30 days after parturition; and

(f) since the beginning of the sampling referred to in point (c)(i), all germinal products of bovine, ovine or caprine origin introduced into or used in the establishment originate from:

(i) establishments free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination; or

(ii) approved germinal product establishments.

2. By way of derogation from point 1, the status free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination may be granted to an establishment if all bovine, ovine or caprine animals originate from establishments free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination, or free with vaccination and have not been vaccinated during the past 3 years, and:

(a) originate from a Member State or a zone free from infection with *Brucella abortus, B. melitensis* and *B. suis* for the relevant animal population;

(b) are entire bovine animals over 12 months of age or entire ovine or caprine animals over 6 months of age and have tested negative in a serological test carried out on a sample taken:

– during the 30 days prior to their introduction into the establishment; or

– during the 30 days following their introduction into the establishment provided they have been kept isolated during this period; or

(c) are post-parturient females kept in isolation since their introduction into the establishment until they tested negative in a serological test carried out on a sample taken not earlier than 30 days after parturition.

3. By way of derogation from point 1, the status free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination may be granted to an establishment with the status free from infection with *Brucella abortus, B. melitensis* and *B. suis* with vaccination if:

(a) the requirements set out in points (a), (b), (d), (e) and (f) of point 1 are fulfilled; and

(b) the requirement set out in point (b)(i) of Section 2 is fulfilled.

SECTION 2
MAINTENANCE OF THE STATUS

The status free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination of an establishment keeping bovine, ovine or caprine animals may only be maintained if:

(a) the requirements set out in points (a), (b), (d), (e) and (f) of point 1 of Section 1 continue to be fulfilled; and

(b) serological testing is carried out with negative results on samples taken from:

(i) all entire bovine animals over 12 months of age and all entire ovine or caprine animals over 6 months of age at appropriate intervals of not more than 12 months determined by the competent authority, taking into account the type of production, the situation of the disease and the identified risk factors; or

(ii) entire bovine animals over 12 months of age and entire ovine or caprine animals over 6 months of age kept in establishments located in a Member State or in a zone free from infection with *Brucella abortus, B. melitensis* and *B. suis*, in accordance with a testing regime set up by the competent authority, taking into account the type of production and the identified risk factors.

SECTION 3
SUSPENSION AND RESTORING OF THE STATUS

1. The status free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination of an establishment keeping bovine, ovine or caprine animals must be suspended if:

(a) one or more of the requirements set out in Section 2 are not fulfilled; or

(b) a case of infection with *Brucella abortus, B. melitensis* and *B. suis* is suspected in a bovine, ovine or caprine animal kept in the establishment.

2. The status free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination may only be restored if:

(a) the requirements set out in points (b), (d), (e) and (f) of point 1 of Section 1 and in point (b) of Section 2 are fulfilled;

(b) the results of further investigations substantiate absence of infection with *Brucella abortus, B. melitensis* and *B. suis* and the status of all suspected cases has been determined.

SECTION 4
WITHDRAWAL AND REGAINING OF THE STATUS

1. The status free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination of an establishment keeping bovine, ovine or caprine animals must be withdrawn if:

(a) one or more of the requirements set out in Section 2 are not fulfilled after the maximum period of time referred to in point (b) of Article 20(3) has lapsed since the status was suspended;

(b) the infection with *Brucella abortus, B. melitensis* and *B. suis* cannot be ruled out in accordance with point 2(b) of Section 3;

(c) a case of infection with *Brucella abortus, B. melitensis* and *B. suis* has been confirmed in a bovine, ovine or caprine animal kept in the establishment; or

(d) it is justified by other needs to control infection with *Brucella abortus*, *B. melitensis*, *B. suis*.

2. If the status free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination has been withdrawn in accordance with point 1(a), it may only be regained if the requirements laid down in Section 2 are fulfilled.

3. If the status free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination has been withdrawn in accordance with point 1(b), 1(c) or 1(d), it may only be regained if all confirmed cases and all animals that have tested non-negative have been removed and the remaining bovine, ovine or caprine animals fulfil the requirements set out in point 1(c) of Section 1.

4. By way of derogation from point 3, where the infection with *B. suis* biovar 2 was confirmed in a single bovine, ovine or caprine animal kept in the establishment, the status may be regained after negative testing was obtained on samples taken in accordance with the requirements set out in point 1(c)(i) of Section 1.

Chapter 2
Establishment free from infection with *Brucella abortus, B. melitensis* and *B. suis* with vaccination

SECTION 1
GRANTING OF THE STATUS

1. The status free from infection with *Brucella abortus, B. melitensis* and *B. suis* with vaccination may only be granted to an establishment keeping bovine, ovine or caprine animals if:

(a) the requirements set out in points (a), (c) and (d) of point 1 of Section 1 of Chapter 1 are fulfilled;

(b) since the beginning of the sampling referred to in point (c)(i) of point 1 of Section 1 of Chapter 1, all bovine, ovine, or caprine animals introduced into the establishment originate from establishments free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination or free from infection with *Brucella abortus, B. melitensis* and *B. suis* with vaccination and:

(i) originate from a Member State or a zone free from infection with *Brucella abortus, B. melitensis* and *B. suis* for the relevant animal population;

(ii) are entire bovine animals over 12 months of age or entire ovine or caprine animals over 6 months of age and have tested negative in a serological test on a sample taken

– during the 30 days prior to their introduction into the establishment; or

– during the 30 days following their introduction into the establishment provided they have been kept isolated during this period; or

(iii) are post-parturient females kept in isolation since their introduction into the establishment until they have tested negative in a serological test carried out on a sample taken not earlier than 30 days after parturition; and

(c) since the beginning of the sampling referred to in point (c)(i) of point 1 of Section 1 of Chapter 1, all germinal products of bovine, ovine or caprine origin introduced into or used in the establishment originate from:

(i) establishments free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination or free from infection with *Brucella abortus, B. melitensis* and *B. suis* with vaccination; or

(ii) approved germinal product establishments.

2. By way of derogation from point 1, the status free from infection with *Brucella abortus, B. melitensis* and *B. suis* with vaccination may be granted to an establishment if all bovine, ovine or caprine animals originate from establishments free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination, or free with vaccination, and:

(a) originate from a Member State or a zone free from infection with *Brucella abortus, B. melitensis* and *B. suis* for the relevant animal population;

(b) are entire bovine animals over 12 months of age or entire ovine or caprine animals over 6 months of age and have tested negative in a serological test carried out on a sample taken:

(i) during the 30 days prior to their introduction into the establishment; or

(ii) during the 30 days following their introduction into the establishment provided they have been kept isolated during this period; or

(c) are post-parturient females kept in isolation since their introduction into the establishment until they have tested negative in a serological test carried out on a sample taken not earlier than 30 days after parturition.

SECTION 2
MAINTENANCE OF THE STATUS

The status free from infection with *Brucella abortus, B. melitensis* and *B. suis* with vaccination of an establishment keeping bovine, ovine or caprine animals may only be maintained if:

(a) the requirements set out in points (b) and (c) of point 1 of Section 1 of this Chapter and in points (a) and (d) of point 1 of Section 1 of Chapter 1 continue to be fulfilled; and

(b) serological testing is carried out with negative results on samples taken from all entire bovine animals over 12 months of age and all entire ovine or caprine animals over 6 months of age at appropriate intervals of not more than 12 months determined by the competent authority taking into account the type of production, the situation of the disease and the identified risk factors.

SECTION 3
SUSPENSION AND RESTORING OF THE STATUS

1. The status free from infection with *Brucella abortus, B. melitensis* and *B. suis* with vaccination of an establishment keeping bovine, ovine or caprine animals must be suspended if:

   (a) one or more of the requirements set out in Section 2 are not fulfilled; or

   (b) a case of infection with *Brucella abortus, B. melitensis* and *B. suis* is suspected in a bovine, ovine or caprine animal kept in the establishment.

2. The status free from infection with *Brucella abortus, B. melitensis* and *B. suis* with vaccination may only be restored if:

   (a) the requirements set out in point 1(d) of Section 1 of Chapter 1 and points (b) and (c) of point 1 of Section 1 and point (b) of Section 2 are fulfilled;

   (b) the results of further investigations substantiate absence of infection with *Brucella abortus, B. melitensis* and *B. suis* and the status of all suspected cases has been determined.

SECTION 4
WITHDRAWAL AND REGAINING OF THE STATUS

1. The status free from infection with *Brucella abortus, B. melitensis* and *B. suis* with vaccination of an establishment keeping bovine, ovine or caprine animals must be withdrawn if:

   (a) one or more of the requirements set out in Section 2 are not fulfilled after the maximum period of time referred to in point (b) of Article 20(3) has lapsed since the status was suspended;

   (b) the infection with *Brucella abortus, B. melitensis* and *B. suis* cannot be ruled out in accordance with point 2(b) of Section 3;

   (c) a case of infection with *Brucella abortus, B. melitensis* and *B. suis* has been confirmed in a bovine, ovine or caprine animal kept in the establishment; or

   (d) it is justified by other needs to control infection with *Brucella abortus*, *B. melitensis*, *B. suis*.

2. If the status free from infection with *Brucella abortus, B. melitensis* and *B. suis* with vaccination has been withdrawn in accordance with point 1(a), it may only be regained if the requirements laid down in Section 2 are fulfilled.

3. If the status free from infection with *Brucella abortus, B. melitensis* and *B. suis* with vaccination has been withdrawn in accordance with point 1(b), 1(c) or 1(d), it may only be regained if all confirmed cases and all animals that have tested non-negative have been removed and the remaining bovine, ovine or caprine animals fulfil the requirements set out in point 1(c) of Section 1 of Chapter 1.

4. By way of derogation from point 3, where the infection with *Brucella suis* biovar 2 was confirmed in a single bovine, ovine or caprine animal kept in the establishment, the status may be regained after negative testing was obtained on samples taken in accordance with the requirements set out in point 1(c)(i) of Section 1 of Chapter 1.

Chapter 3
Member State or zone free from infection with *Brucella abortus, B. melitensis* and *B. suis* as regards kept bovine animals

SECTION 1
GRANTING OF THE STATUS AS REGARDS KEPT BOVINE ANIMALS

The status free from infection with *Brucella abortus, B. melitensis* and *B. suis* as regards kept bovine animals may only be granted to a Member State or a zone if:

(a) for at least the past 3 years there has been no confirmed case of infection with *Brucella abortus, B. melitensis* and *B. suis* in kept bovine animals;

(b) general surveillance requirements have been carried out for the past 3 years in accordance with point (a) of Article 3(1) for the early detection of infection with *Brucella abortus, B. melitensis* and *B. suis* in kept bovine animals, which included at least:

  (i) the regular submission of samples from abortion cases for laboratory testing;

  (ii) the timely investigation of abortion cases that may have been caused by infection with *Brucella abortus*, *B. melitensis* and *B. suis*;

(c) during the past 3 years, at least 99.8 % of the establishments keeping bovine animals, representing at least 99.9 % of the bovine population, have maintained their status free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination;

(d) vaccination of bovine animals against *Brucella abortus, B. melitensis* and *B. suis* has not taken place at least for the past 3 years and no bovine animal introduced into the Member State or zone has been vaccinated during the past 3 years prior to its introduction.

SECTION 2
MAINTENANCE OF THE STATUS AS REGARDS KEPT BOVINE ANIMALS

1. The status free from infection with *Brucella abortus, B. melitensis* and *B. suis* as regards kept bovine animals of a Member State or a zone may only be maintained if:

  (a) the requirements set out in points (a), (b) and (d) of Section 1 continue to be fulfilled; and

  (b) for the first 2 consecutive years following granting of the status, annual surveillance based on a representative sample of all establishments keeping bovine animals has been carried out that must allow at least for the detection, with a 95 % level of confidence, of infection with *Brucella abortus, B. melitensis* and *B. suis,* at a target prevalence rate of 0.2 % of the establishments keeping bovine animals or a target prevalence rate of 0.1 % of the bovine population;

(c) if no case of infection with *Brucella abortus, B. melitensis* and *B. suis* has been confirmed in kept bovine animals for 2 consecutive years following granting of the status, surveillance must be based on:

(i) random annual surveillance that must allow at least for the detection, with a 95 % level of confidence, of infection with *Brucella abortus, B. melitensis* and *B. suis*, at a target prevalence rate of 0.2 % of the establishments keeping bovine animals or a target prevalence rate of 0.1 % of the bovine population; or

(ii) risk-based annual surveillance to detect infection with *Brucella abortus, B. melitensis* and *B. suis* taking into account the systems of production and the risk factors identified, including spread of infection from other animals than kept bovine animals.

2. The status of a Member State or a zone free from infection with *Brucella abortus, B. melitensis* and *B. suis* as regards kept bovine animals is not affected by the confirmation of infection of *Brucella abortus, B. melitensis* and *B. suis* in an animal population other than kept bovine animals provided that effective measures have been implemented, and are periodically assessed, to prevent transmission of infection with *Brucella abortus, B. melitensis* and *B. suis* to kept bovine animals.

3. By way of derogation from point 1(a), the status free from infection with *Brucella abortus, B. melitensis* and *B. suis* as regards kept bovine animals of a Member State or a zone may be maintained in the event of the confirmation of a case of infection with *Brucella abortus, B. melitensis* and *B. suis* if:

(a) the establishment in which the infection with *Brucella abortus, B. melitensis* and *B. suis* was detected in kept bovine animals has been immediately subjected to the relevant disease control measures laid down in Article 24;

(b) within 60 days after the first confirmation of the infection, the competent authority has conducted an epidemiological enquiry and investigations, as laid down in Article 25, to identify the likely source and the distribution of the infection and established conclusions on the likely source of infection and only a limited number of establishments were infected and those establishments are epidemiologically linked to the first detected outbreak;

(c) the relevant disease control measures laid down in Article 21 or Article 24 have been immediately implemented in each establishment identified with suspected or confirmed cases following implementation of the measures provided for in point (b) until their disease-free status is restored or regained;

(d) the surveillance referred to in point 1 has been adapted and has demonstrated that the incident has been resolved.

Chapter 4
Member State or zone free from infection with *Brucella abortus, B. melitensis* and *B. suis* as regards kept ovine and caprine animals

SECTION 1
GRANTING OF THE STATUS AS REGARDS KEPT OVINE AND CAPRINE ANIMALS

The status free from infection with *Brucella abortus, B. melitensis* and *B. suis* as regards kept ovine and caprine animals may only be granted to a Member State or a zone if:

(a) for at least the past 3 years there has been no confirmed case of infection with *Brucella abortus, B. melitensis* and *B. suis* in kept ovine and caprine animals;

(b) general surveillance requirements have been carried out for the past 3 years in accordance with point (a) of Article 3(1) for the early detection of infection with *Brucella abortus, B. melitensis* and *B. suis* in kept ovine and caprine animals, which included at least:

  (i) the regular submission of samples from abortion cases for laboratory testing;

  (ii) the timely investigation of abortion cases that may have been caused by infection with *Brucella abortus*, *B. melitensis* and *B. suis*;

(c) during the past 3 years, surveillance has been carried out on the ovine and caprine population and at least 99.8 % of the establishments keeping ovine or caprine animals, representing at least 99.9 % of the ovine and caprine population, have maintained their status free from infection with *Brucella abortus, B. melitensis* and *B. suis* without vaccination; and

(d) vaccination of ovine and caprine animals against *Brucella abortus, B. melitensis* and *B. suis* has not taken place for at least the past 3 years and no ovine or caprine animal introduced into the Member State or zone has been vaccinated during the past 3 years prior to introduction.

SECTION 2
MAINTENANCE OF THE STATUS AS REGARDS KEPT OVINE AND CAPRINE ANIMALS

1. The status free from infection with *Brucella abortus, B. melitensis* and *B. suis* as regards kept ovine and caprine animals of a Member State or a zone may only be maintained if:

  (a) the requirements defined in points (a), (b) and (d) of Section 1 continue to be fulfilled; and

  (b) for the first 2 consecutive years following granting of the status, annual surveillance based on a representative sample of all establishments where ovine or caprine animals are kept shall be carried out that must allow at least for the detection, with a 95 % level of confidence, of infection with *Brucella abortus, B. melitensis* and *B. suis* at a target prevalence rate of 0.2 % of the establishments keeping ovine or caprine animals or a target prevalence rate of 0.1 % of the ovine and caprine population;

(c) if no case of infection with *Brucella abortus, B. melitensis* and *B. suis* has been confirmed in kept ovine and caprine animals for 2 consecutive years following granting of the status, surveillance must be based on:

(i) random annual surveillance that must allow at least for the detection, with a 95 % level of confidence, of infection with *Brucella abortus, B. melitensis* and *B. suis* at a target prevalence rate of 0.2 % of the establishments keeping ovine or caprine animals or a target prevalence rate of 0.1 % of the ovine and caprine population; or

(ii) risk-based annual surveillance to detect infection with *Brucella abortus, B. melitensis* and *B. suis*, which takes into account the systems of production and the risk factors identified, including spread of infection from other animals than kept ovine and caprine animals.

2. The status of a Member State or a zone free from infection with *Brucella abortus, B. melitensis* and *B. suis* as regards kept ovine and caprine animals is not affected by the confirmation of infection of *Brucella abortus, B. melitensis* and *B. suis* in an animal population other than kept ovine and caprine animals provided that effective measures have been implemented, and are periodically assessed, to prevent transmission of infection with *Brucella abortus, B. melitensis* and *B. suis* to kept ovine and caprine animals.

3. By way of derogation from point 1(a), the status free from infection with *Brucella abortus, B. melitensis* and *B. suis* as regards kept ovine and caprine animals of a Member State or a zone may be maintained in the event of the confirmation of a case of infection with *Brucella abortus, B. melitensis* and *B. suis* if:

(a) the establishment in which the infection with *Brucella abortus, B. melitensis* and *B. suis* was detected in kept ovine and caprine animals has been immediately subjected to the relevant disease control measures laid down in Article 24;

(b) within 60 days after the first confirmation of the infection, the competent authority has conducted an epidemiological enquiry and investigations, as laid down in Article 25, to identify the likely source and the distribution of the infection and established conclusions on the likely source of infection and only a limited number of establishments were infected and those establishments are epidemiologically linked to the first detected outbreak;

(c) the relevant disease control measures laid down in Article 21 or Article 24 have been immediately implemented in each establishment identified with suspected or confirmed cases following implementation of the measures provided for in point (b) until their disease-free status is restored or regained; and

(d) the surveillance referred to in point 1 has been adapted and has demonstrated that the incident has been resolved.

Part II
Infection with *Mycobacterium tuberculosis* complex

Chapter 1
Establishment free from infection with *Mycobacterium tuberculosis* complex

SECTION 1
GRANTING OF THE STATUS

1. The status free from infection with *Mycobacterium tuberculosis* complex (*Mycobacterium bovis, Mycobacterium tuberculosis, Mycobacterium caprae)* (MTBC) may only be granted to an establishment keeping bovine animals if:

   (a) during the past 12 months there has been no confirmed case of infection with MTBC in bovine animals kept in the establishment;

   (b) the bovine animals over 6 weeks of age present in the establishment at the time of testing or sampling have tested negative to immunological test on two occasions as follows:

      (i) the first test must be carried out on bovine animals or samples taken from bovine animals not earlier than 6 months after the removal of the last confirmed case and of the last animal that tested positive in an immunological test;

      (ii) the second test must be carried out on bovine animals or on samples taken from bovine animals not earlier than 6 months and not later than 12 months following the date of testing of the bovine animal or taking of the samples referred to in point (i);

   (c) since the beginning of the testing or sampling referred to in point (b)(i), all bovine animals introduced into the establishment originate from establishments free from infection with MTBC and:

      (i) originate from a Member State or a zone free from infection with MTBC;

      (ii) are bovine animals over 6 weeks of age and have tested negative in an immunological test:

         – during the 30 days prior to their introduction into the establishment; or

         – during the 30 days after their introduction provided they have been kept isolated during this period; and

   (d) since the beginning of the testing or sampling referred to in point (b)(i), all germinal products of bovine origin introduced into or used in the establishment originate from:

      (i) establishments free from infection with MTBC*;* or

      (ii) approved germinal product establishments.

2. By way of derogation from point 1, the status free from infection with MTBC may be granted to an establishment if all bovine animals originate from establishments free from infection with MTBC and:

(a) originate from a Member State or a zone free from infection with MTBC;

(b) if they are bovine animals over 6 weeks of age, they have tested negative to an immunological test:

(i) during the 30 days prior to their introduction into the establishment; or

(ii) during the 30 days after their introduction provided they have been kept in isolation during this period.

3. By way of derogation from points 1(c) and 2(b), the competent authority may not require the test if:

(a) the bovine animals introduced into the establishment:

(i) have tested negative in an immunological test carried out during the past 6 months; and

(ii) originate from establishments where the bovine animals have tested negative to a testing regime as provided for in points 1(c) or 2 of Section 2 carried out during the past 6 months; or

(b) the bovine animals introduced into the establishment:

(i) have tested negative in an immunological test carried out during the past 12 months; and

(ii) originate from establishments where the bovine animals have tested negative to a testing regime as provided for in point 2(b) or 2 (c) of Section 2 carried out during the past 12 months.

SECTION 2
MAINTENANCE OF THE STATUS

1. The status free from infection with MTBC of an establishment keeping bovine animals may only be maintained if:

(a) the requirements set out in points (a), (c) and (d) of point 1 of Section 1 continue to be fulfilled;

(b) any suspected case of infection with MTBC in a bovine animal kept on that establishment or introduced from that establishment into a slaughterhouse is notified to the competent authority and investigated; and

(c) an immunological test has been carried out, with negative results, on all bovine animals over 6 weeks of age, at intervals of not more than 12 months.

2. By way of derogation from point 1(c), the competent authority may modify the testing regime as follows:

(a) in a Member State or in a zone where the annual percentage, calculated on 31 December of each year, of establishments infected with MTBC is not more than 1 % during the last 24 months, the interval between tests may be extended to 24 months;

(b) in a Member State or in a zone where the annual percentage, calculated on 31 December of each year, of establishments infected with MTBC is not more than 0.2 % for the last 48 months, the interval between tests may be extended to 36 months;

(c) in a Member State or in a zone where the annual percentage, calculated on 31 December of each year, of establishments infected with MTBC is not more than 0.1 % for the last 72 months, the interval between tests may be extended to 48 months;

(d) in a Member State or a zone free from infection with MTBC, if the risk of transmission of MTBC from wild animals to bovine animals has been assessed by appropriate surveillance, the interval between tests may be based on the type of production and the risk factors identified, taking into account at least the following risks:

(i) a location associated with suspected or confirmed infection with MTBC in wild animals;

(ii) a history of infection with MTBC within the last 5 years;

(iii) an epidemiological link with establishments in any of points (i) or (ii).

SECTION 3
SUSPENSION AND RESTORING OF THE STATUS

1. The status free from infection with MTBC of an establishment keeping bovine animals must be suspended if:

(a) one or more of the requirements laid down in Section 2 are not fulfilled; or

(b) a case of infection with MTBC is suspected in a bovine animal kept in the establishment.

2. The status free from infection with MTBC may only be restored, if:

(a) the requirements laid down in points 1(c) and 1(d) of point 1 of Section 1, 1(b), of Section 2 and, as relevant, in point 1(c) or in point 2 of Section 2 are fulfilled;

(b) the results of further investigations substantiate absence of infection with MTBC and the status of all suspected cases has been determined. In case, suspected bovine animals are slaughtered in that context, investigations must include examination of samples with direct diagnostic methods.

SECTION 4
WITHDRAWAL AND REGAINING OF THE STATUS

1. The status free from infection with MTBC of an establishment keeping bovine animals must be withdrawn if:

(a) one or more of the requirements laid down in Section 2 are not fulfilled after the maximum period of time referred to in point (b) of Article 20(3) has lapsed since the status was suspended;

(b) the infection with MTBC cannot be ruled out in accordance with point 2(b) of Section 3;

(c) a case of infection with MTBC has been confirmed in a bovine animal kept in the establishment; or

(d) it is justified by other needs to control infection with MTBC.

2. If the status free from infection with MTBC has been withdrawn in accordance with point 1(a), it may only be regained if the requirements laid down in Section 2 are fulfilled.

3. If the status free from infection with MTBC has been withdrawn in accordance with point 1(b), 1(c) or 1(d), it may only be regained if:

(a) all confirmed cases and all animals that have tested non negative in a immunological test have been removed; and

(b) the remaining bovine animals fulfil the requirements set out in point 1(b) of Section 1.

4. By way of derogation from point 3(b), the status may be regained if:

(a) all bovine animals over 6 weeks of age present in the establishment at the time of testing have tested negative in two immunological tests as follows:

(i) the first test must be carried out on bovine animals or samples taken from bovine animals not earlier than 2 months after the removal of the last confirmed case and of the last animal that tested positive in an immunological test;

(ii) the second test must be carried out on bovine animals or on samples taken from bovine animals not earlier than 2 months and not later than 12 months following the date of testing or sampling of the bovine animal as referred to in point (i); and

(b) at least one of the following conditions apply:

(i) the conclusion of the epidemiological enquiry indicates that the infection is due to the introduction of one or more infected animals into the establishment during the past 12 months prior to the detection of the infection with MTBC; or

(ii) only a single case was confirmed or only a single bovine animal tested positive in an immunological test for MTBC since the detection of the infection with MTBC, and the status of the establishment has not been withdrawn during the past 3 years; or

(iii) bovine animals in the establishment have tested negative in an immunological test carried out less than 12 months prior to the detection of the infection with MTBC in accordance with point 1(c) or 2 of Section 2.

Chapter 2
Member State or zone free from infection with MTBC

SECTION 1
GRANTING OF THE STATUS AS REGARDS KEPT BOVINE ANIMALS

The status free from infection with MTBC as regards kept bovine animals may only be granted to a Member State or a zone if:

(a) during the past 3 years at least 99.8 % of the establishments keeping bovine animals, representing at least 99.9 % of the bovine population, have maintained their status free from infection with MTBC and the incidence rate of establishments confirmed infected during the year did not exceed 0.1 %; and

(b) general surveillance requirements have been carried out for the past 3 years in accordance with point (a) of Article 3(1) for the detection of infection with MTBC in kept bovine animals and included at least:

  (i) the systematic research of lesions of infection with MTBC in all bovine animals slaughtered through ante- and post-mortem surveillance;

  (ii) the investigations of lesions that could be due to infection with MTBC.

SECTION 2
MAINTENANCE OF THE STATUS

1. The status free from infection with MTBC as regards kept bovine animals of a Member State or a zone may only be maintained if:

  (a) the requirements in point (b) of Section 1 continue to be fulfilled; and

  (b) for the first 2 consecutive years following granting of the status random annual surveillance based on a representative sampling of all establishments where bovine animals are kept must be carried out to demonstrate with a 95 % level of confidence, that:

    (i) at least 99.8 % of the establishments, representing at least 99.9 % of the bovine population are free from infection with MTBC;

    (ii) the incidence rate of establishment confirmed infected during the year does not exceed 0.1 %;

  (c) if the conditions in point (b) were fulfilled for 2 consecutive years, surveillance is based on:

    (i) random annual surveillance to demonstrate at least with a confidence level of 95 %, that the incidence rate of establishments confirmed infected during the year does not exceed 0.1 %; or

    (ii) risk-based annual surveillance carried out to detect infection with MTBC, taking into account the systems of production, the risk factors identified, including the spread of infection from other animals than kept bovine animals and increased surveillance in establishments associated with at

least one of the specific risks referred to in point 2(d) of Section 2 of Chapter 1.

2. The status of a Member State or a zone free from infection with MTBC is not affected by the confirmation of infection with MTBC in the animal population other than kept bovine animals, provided that effective measures have been implemented, and are periodically assessed, to prevent transmission of infection with MTBC to kept bovine animals.

Part III
Enzootic bovine leukosis

Chapter 1
Establishment free from enzootic bovine leukosis

SECTION 1
GRANTING OF THE STATUS

1. The status free from enzootic bovine leukosis (EBL) may only be granted to an establishment keeping bovine animals if:

   (a) during the past 24 months there has been no confirmed case of EBL in bovine animals kept in the establishment;

   (b) during the past 12 months, bovine animals older than 24 months of age kept in the establishment have tested negative to a serological test, on at least two occasions at an interval of not less than 4 months;

   (c) since the beginning of the sampling referred to in point (b), all bovine animals introduced into the establishment:

      (i) originate from establishments free from EBL; or

      (ii) originate from establishments where there has been no evidence of EBL either clinical, post-mortem, or as a result of a diagnostic test for EBL within the 24 months prior to their dispatch; and

         – if over 24 months of age,

            they have been subjected to serological tests, with negative results, on two occasions at an interval of not less than 4 months while kept in isolation from other bovine animals of the establishment; or

            they have been subjected to a serological test, with a negative result, within 30 days prior to their introduction provided all bovine animals have been tested in accordance with point (b);

         – if less than 24 months of age,

            they were born to dams, that have been subjected to a serological test for EBL, with negative results, carried out on samples taken during the past 12 months on two occasions at an interval of not less than 4 months; and

   (d) since the beginning of the sampling referred to in point (b), all germinal products of bovine animals introduced into the establishment originate from:

      (i) establishments free from EBL; or

      (ii) approved germinal product establishments.

2. By way of derogation from point 1, the status free from EBL may be granted to an establishment if all bovine animals originate from establishments free from EBL located either in a Member State or zone free from EBL or in a Member State or zone covered by an approved eradication programme.

SECTION 2
MAINTENANCE OF THE STATUS

The status free from EBL of an establishment keeping bovine animals may only be maintained if:

(a) the requirements laid down in points (a), (c) and (d) of point 1 of Section 1 continue to be fulfilled; and

(b) serological testing for EBL is carried out, with negative results, on samples taken

  (i) at intervals of not more than 36 months from all bovine animals over 24 months of age; or

  (ii) in accordance with point (b) or (c) of Section 2 of Chapter 2, as relevant, if the establishment is located in a Member State or zone free from EBL.

SECTION 3
SUSPENSION AND RESTORING OF THE STATUS

1. The status free from EBL of an establishment keeping bovine animals must be suspended if:

  (a) one or more of the requirements laid down in Section 2 are not fulfilled;

  (b) a case of EBL in a bovine animal that is kept on the establishment is suspected.

2. The status free from EBL may only be restored if:

  (a) the requirements laid down in points (c) and (d) of point 1 of Section 1 and point (b) of Section 2 are fulfilled;

  (b) the results of further investigations substantiate absence of EBL and the status of all suspected cases has been determined.

SECTION 4
WITHDRAWAL AND REGAINING OF THE STATUS

1. The status free from EBL of an establishment keeping bovine animals must be withdrawn if:

  (a) one or more of the requirements laid down in Section 2 are not fulfilled after the maximum period of time referred to in point (b) of Article 20(3) has lapsed since the status was suspended; or

  (b) a case of EBL has been confirmed in a bovine animal kept in the establishment.

2. If the status free from EBL has been withdrawn in accordance with point 1(a), it may only be regained if the requirements laid down in points (c) and (d) of point 1 of Section 1 and point (b) of Section 2 are fulfilled.

3. If the status free from EBL has been withdrawn in accordance with point 1(b), it may only be regained if:

  (a) any bovine animal presenting a positive test result for EBL and all of their offspring younger than 24 months of age have been removed;

(b) all bovine animals over 12 months of age have been tested negative in a serological test, on two occasions at an interval of not less than 4 months, where the first test must be carried out on samples not taken earlier than 4 months after the removal of the last confirmed case.

4. By way of derogation from point (3)(a), the offspring of dams that have been tested positive in a serological test for EBL or which have shown lesions of EBL may be kept in the establishment if:

(a) they have been separated from the dam immediately after calving and tested negative in a PCR test, on two occasions, where the first sample must be taken within the period of 3 to 5 weeks and the second within 8 to 10 weeks postpartum; and

(b) they remain in the establishment until they are 24 months of age and are tested negative in a serological test, or they are sent before that test directly to the slaughterhouse in accordance with the requirements laid down in Article 27(4).

Chapter 2
Member State or zone free from EBL

SECTION 1
GRANTING OF THE STATUS

The status free from EBL as regards kept bovine animals may only be granted to a Member State or a zone if:

(a) at least 99.8 % of the bovine establishments are free from EBL; and

(b) all bovine animals over 24 months of age slaughtered within this Member State or zone are subjected to an official post-mortem examination with samples from all animals with tumours that could be caused by EBL being subjected to laboratory examination to confirm or rule out the presence of EBL.

SECTION 2
MAINTENANCE OF THE STATUS

The status free from EBL as regards kept bovine animals of a Member State or a zone may only be maintained if:

(a) the requirements set out in Section 1 continue to be fulfilled; and

(b) during the first 5 years after the granting of the status free from EBL, surveillance is carried out based on:

(i) annual random sampling to detect at least, with a 95 % level of confidence, establishments infected with EBL at a target prevalence rate of 0.2 %; or

(ii) serological testing of all bovine animals over 24 months of age on at least one occasion;

(c) following the first 5 years after the granting of the status free from EBL, surveillance is carried out to demonstrate the absence of infection, taking into account the systems of production and the risk factors identified.

Part IV
Infectious bovine rhinotracheitis / infectious pustular vulvovaginitis

Chapter 1
Establishment free from infectious bovine rhinotracheitis / infectious pustular vulvovaginitis

SECTION 1
GRANTING OF THE STATUS

1. The status free from infectious bovine rhinotracheitis / infectious pustular vulvovaginitis (IBR/IPV) may only be granted to an establishment keeping bovine animals if:

   (a) during the past 12 months there has been no confirmed case of IBR/IPV in bovine animals kept in the establishment;

   (b) during the past 2 years none of the bovine animals kept in the establishment has been vaccinated against IBR/IPV;

   (c) the bovine animals kept in the establishment have been subjected to at least one of the following testing regimes taking into account previous DIVA vaccinations, where serological tests for the detection of antibodies against whole BoHV-1 or, if necessary, antibodies against BoHV-1-gE have been carried out on:

      (i) a blood, milk or meat juice sample taken from each bovine animal over a period of not more than 12 months; or

      (ii) blood, milk or meat juice samples taken on at least two occasions at an interval of not less than 2 months and not more than 12 months from

         – all female bovine animals over 12 months of age, and

         – all male bovine animals used or intended for breeding over 12 months of age, and

         – a random sample of male animals not intended for breeding over 12 months of age. The number of animals tested must allow at least for the detection, with a 95 % level of confidence, of seropositive animals at a target prevalence rate of 10 %; or

      (iii) in the case of an establishment in which at least 30 % of the bovine animals are lactating,

         – bulk milk samples taken on at least three occasions at intervals of not less than 3 months from lactating female bovine animals representing all epidemiological units of the establishment, and

         – blood samples taken from all non-lactating female bovine animals over 12 months of age, and from all male bovine animals used or intended for breeding over 12 months of age, and

– a random blood or meat juice sample taken from male bovine animals not intended for breeding older than 12 months of age. The number of animals tested must allow at least for the detection, with a 95 % level of confidence, of seropositive animals at a target prevalence rate of 10 %; or

(iv) in the case of an establishment in which less than 5 % of the kept bovine animals are male and at least 95 % of the female animals over 24 months are intended or used for milk production, bulk milk samples taken on at least six occasions at intervals of not less than 2 months from lactating female bovine animals representing all epidemiological units of the establishment;

(d) since the beginning of the sampling referred to in point (c) all bovine animals introduced into the establishment:

(i) originate from establishments free from IBR/IPV and, in case the establishments of origin are located in a Member State or zone that is neither free from IBR/IPV nor covered by an approved eradication programme, have tested negative in a serological test for the detection of antibodies against whole BoHV-1 or, if necessary, antibodies against BoHV-1-gE on a sample taken after their introduction and before the granting of the status free from IBR/IPV; or

(ii) have been subjected to quarantine prior to their introduction and have tested negative in serological test for the detection of antibodies against whole BoHV-1 on a sample taken not earlier than 21 days after the beginning of the quarantine; and

(e) since the beginning of the sampling referred to in point (c) all germinal products of bovine animals introduced into the establishment originate from:

(i) establishments free from IBR/IPV; or

(ii) approved germinal product establishments.

2. By way of derogation from point 1, the status free from IBR/IPV may be granted to an establishment if all bovine animals originate from establishments free from IBR/IPV located either in a Member State or zone free from IBR/IPV or in a Member State or zone under an approved eradication programme, provided they fulfil the requirements set out in points (c) and (d) of Section 2, as relevant.

SECTION 2
MAINTENANCE OF THE STATUS

The status free from IBR/IPV may only be maintained in an establishment keeping bovine animals if:

(a) the requirements laid down in points (a), (b) and (e) of point 1 of Section 1 continue to be fulfilled;

(b) serological testing for the detection of antibodies against whole BoHV-1 or, if necessary, antibodies against BoHV-1-gE is carried out taking into account previous vaccinations with a DIVA vaccine, with negative results,

(i) on blood, milk or meat juice samples taken annually from all bovine animals older than 24 months of age; or

(ii) in the case of an establishment, in which at least 30 % of the bovine animals are lactating, at least annually on:

– bulk milk samples taken on at least three occasions at intervals of not less than 3 months from lactating female bovine animals representing all epidemiological units of the establishment, and

– blood samples taken from all breeding male bovine animals older than 24 months of age; or,

(iii) in the case of an establishment, in which less than 5 % of the kept bovine animals are male and at least 95 % of the female animals over 24 months are intended or used for milk production, at least annually on bulk milk samples taken on at least six occasions at intervals of not less than 2 months from lactating female bovine animals representing all epidemiological units of the establishment; or

(iv) provided the status free from IBR/IPV has been maintained for the past 3 consecutive years, annually on blood or milk samples taken from a number of bovine animals that must allow at least for the detection, with a 95 % level of confidence, of seropositive animals at a target prevalence rate of 10 %; or

(v) if the establishment is located in a Member State or zone free from IBR/IPV, on samples taken in accordance with point 1(b) of Section 2 of Chapter 2 or point 3 of Section 2 of Chapter 2, if relevant.

(c) only bovine animals that have not been vaccinated against infection with IBR/IPV are introduced into the establishment if it is located in a Member State or zone:

(i) free from IBR/IPV; or

(ii) where a vaccination ban is in place as part of the eradication strategy under an approved eradication programme.

(d) all bovine animals that are introduced fulfil the requirements laid down in point 1(d)(ii) of Section 1 or originate from establishments free from IBR/IPV and have tested negative in a serological test for the detection of antibodies against whole BoHV-1 or, if necessary, antibodies against BoHV-1-gE on a sample taken in the establishments of origin within 15 days prior to their dispatch, in cases where:

(i) the establishment is located in a Member State or zone free from IBR/IPV and the establishments of origin are not located in a Member State or zone free from IBR/IPV; or

(ii) the establishment is located in a Member State or zone covered by an approved eradication programme and the establishments of origin are located in a Member State or zone that is neither free from IBR/IPV nor covered by an approved eradication programme.

SECTION 3
SUSPENSION AND RESTORING OF THE STATUS

1. The status free from IBR/IPV of an establishment keeping bovine animals must be suspended if:

(a) one or more of the requirements laid down in Section 2 are not fulfilled;

(b) a case of IBR/IPV is suspected in a bovine animal kept in the establishment.

2. The status free from IBR/IPV may only be restored if:

(a) the requirements laid down in points 1 (b) and (e) of Section 1 and points (b), (c) and (d) of Section 2 are fulfilled;

(b) the results of further investigations substantiate absence of IBR/IPV and the status of all suspected cases has been determined.

SECTION 4
WITHDRAWAL AND REGAINING OF THE STATUS

1. The status free from IBR/IPV of an establishment keeping bovine animals must be withdrawn if:

(a) one or more of the requirements laid down in Section 2 are not fulfilled after the maximum period of time referred to in point (b) of Article 20(3) has lapsed since the status was suspended;

(b) a case of IBR/IPV has been confirmed in a bovine animal kept in the establishment.

2. If the status free from IBR/IPV has been withdrawn in accordance with point 1(a), it may only be regained if the requirements laid down in points (b) and (e) of point 1 of Section 1 and points (b), (c) and (d) of Section 2 are fulfilled.

3. If the status free from IBR/IPV has been withdrawn in accordance with point 1(b), it may only be regained if:

(a) all confirmed cases have been removed;

(b) at least one of the testing regimes laid down in point 1(c) of Section 1 has been carried out with negative results on samples that have been taken not earlier than 30 days after the removal of the last confirmed case.

Chapter 2
Member State or zone free from IBR/IPV

SECTION 1
GRANTING OF THE STATUS

The status free from IBR/IPV as regards kept bovine animals may only be granted to a Member State or a zone if

(a) vaccination against IBR/IPV has been prohibited for kept bovine animals; and

(b) at least 99.8 % of the establishments representing at least 99.9 % of the corresponding bovine population are free from IBR/IPV.

SECTION 2
MAINTENANCE OF THE STATUS

1. The status free from IBR/IPV as regards kept bovine animals of a Member State or a zone may only be maintained if:

(a) the requirements laid down in Section 1 continue to be fulfilled; and

(b) surveillance is carried out annually based on random sampling that must allow at least for the detection, with a 95 % level of confidence, of the infection of establishments with BoHV-1 at a target prevalence rate of 0.2 % of the establishments or of BoHV-1 infected bovine animals with a target prevalence rate of 0.1 % of the bovine population.

2. By way of derogation from point 1(a), the use of DIVA vaccination may be authorised by the competent authority in the event of an outbreak, if:

(a) the result of the epidemiological enquiry and investigations according to Article 25 has demonstrated that only a limited number of establishments were involved in the outbreak;

(b) its use is limited to controlling this outbreak as deemed necessary by the competent authority;

(c) the bovine animals are DIVA vaccinated under the supervision of the competent authority and the use of DIVA vaccines is documented for each animal;

(d) the DIVA vaccinated bovine animals are only moved directly to a slaughterhouse or to an establishment in another zone or Member State where no vaccination ban is in place.

3. By way of derogation from point 1(b), surveillance may be carried out to demonstrate yearly the absence of infection with BoHV-1 taking into account the systems of production and the risk factors identified, provided no outbreaks have been detected for 5 consecutive years following the granting of the status free from IBR/IPV in this Member state or zone.

Part V
Infection with Aujeszky's disease virus

Chapter 1
Establishment free from infection with Aujeszky's disease virus

SECTION 1
GRANTING OF THE STATUS

1. The status free from infection with Aujeszky's disease virus (ADV) may only be granted to an establishment keeping porcine animals if:

   (a) during the past 12 months there has been no confirmed case of infection with ADV in porcine animals kept in the establishment;

   (b) during the past 12 months none of the porcine animals kept in the establishment has been vaccinated against AD;

   (c) during the past 12 months, the porcine animals kept in the establishment have been subjected to one of the following testing regimes taking into account previous DIVA vaccinations where serological tests for the detection of antibodies against ADV or, if necessary, antibodies against ADV-gE have been carried out, with negative results, on:

      (i) a blood or meat juice sample taken from each porcine animal; or

      (ii) blood or meat juice samples taken on two occasions at an interval of 2 to 3 months from a number of animals that must allow at least for the detection, with a 95 % level of confidence, of seropositive animals at a target prevalence rate of 10 %.

   (d) since the beginning of the sampling referred to in point (c), all porcine animals introduced into the establishment:

      (i) originate from establishments free from infection with ADV and, in case the establishments of origin are located in a Member State or zone that is neither free from infection with ADV nor covered by an approved eradication programme, have tested negative in a serological test for the detection of antibodies against whole ADV or, if necessary, antibodies against ADV-gE after their introduction and before the granting of the status free from infection with ADV; or

      (ii) have been subjected to quarantine for a period of at least 30 days prior to their introduction and have tested negative in a serological test for the detection of antibodies against whole ADV on two occasions at an interval of not less than 30 days between collection of each sample. The sample for the last test must be taken within 15 days prior to dispatch.

   (e) since the beginning of the sampling referred to in point (c) all germinal products from porcine animals introduced into the establishment originate from:

      (i) establishments free from infection with ADV; or

(ii) approved germinal product establishments.

2. By way of derogation from point 1, the status free from infection with ADV may be granted to an establishment if all porcine animals originate from establishments free from infection with ADV located either in a Member State or zone free from infection with ADV or in a Member State or zone covered by an approved eradication programme, provided they fulfil the requirements set out in point (d) of Section 2.

SECTION 2
MAINTENANCE OF THE STATUS

The status free from infection with ADV of an establishment keeping porcine animals may only be maintained if:

(a) the requirements laid down in points (a), (b) and (e) of point 1 of Section 1 continue to be fulfilled;

(b) serological testing is carried out, with negative results, on a representative number of blood or meat juice samples taken from the porcine animals kept in the establishment, to verify the absence of infection with ADV based on a testing regime that takes into account the production cycle and the risk of introduction of ADV:

(i) at least once a year, in case all kept porcine animals are not vaccinated against AD, with tests for the detection of antibodies against whole ADV; or

(ii) at least twice a year, with tests for the detection of antibodies against whole ADV and tests for the detection of antibodies against ADV-gE, if necessary;

(c) provided the establishment is located in a Member State or zone free from infection with ADV, the serological testing referred to in point (b) is carried out, as required, in accordance with the surveillance provided for in point 1(b) of Section 2 of Chapter 2 or point 4 of Section 2 of Chapter 2, if relevant;

(d) all porcine animals, that are introduced:

(i) fulfil the requirements set out in point 1(d)(ii) of Section 1; or

(ii) originate from establishments free from infection with ADV and have been subjected to a serological test for antibodies against whole ADV, with a negative result, on a sample collected in the establishments of origin within 15 days prior to their dispatch, in cases where:

– the establishment is located in a Member State or zone free from infection with ADV and the establishments of origin are not located in a Member State or zone free from infection with ADV; or

– the establishment is located in a Member State or zone covered by an approved eradication programme and the establishments of origin are located in a Member State or zone that is neither free from infection with ADV nor covered by an approved eradication programme.

The number of porcine animals tested must allow at least for the detection, with a 95 % level of confidence, of seropositive animals at a target prevalence rate of 10 %.

By way of derogation from the first subparagraph, for porcine animals less than 4 months old born to DIVA-vaccinated dams the serological test for the detection of antibodies against ADV-gE may be used.

SECTION 3
SUSPENSION AND RESTORING OF THE STATUS

1. The status free from infection with ADV of an establishment keeping porcine animals must be suspended if:
   (a) one or more of the requirements laid down in Section 2 are no longer fulfilled;
   (b) a case of infection with ADV is suspected in a porcine animal kept in the establishment.
2. The status free from infection with ADV may only be restored if:
   (a) the requirements laid down in points (b) and (e) of point 1 of Section 1 and point (b) or (c), if relevant, and (d) of Section 2 are fulfilled;
   (b) the results of further investigations substantiate absence of infection with ADV and the status of all suspected cases has been determined.

SECTION 4
WITHDRAWAL AND REGAINING OF THE STATUS

1. The status free from infection with ADV of an establishment keeping porcine animals must be withdrawn if:
   (a) one or more of the requirements laid down in Section 2 are not fulfilled after the maximum period of time referred to in point (b) of Article 20(3) has lapsed since the status was suspended;
   (b) a case of infection with ADV has been confirmed in a porcine animal kept in the establishment.
2. If the status free from infection with ADV has been withdrawn in accordance with point 1(a), it may only be regained if the requirements set out in points (b) and (e) of point 1 of Section 1 and point (b) or (c), if relevant, and (d) of Section 2 are fulfilled.
3. If the status free from infection with ADV has been withdrawn in accordance with point 1(b), it may only be regained, if all porcine animals of the establishment have been removed.

Chapter 2
Member State or zone free from infection with Aujeszky's disease virus

SECTION 1
GRANTING OF THE STATUS

The status free from infection with ADV as regards kept porcine animals may only be granted to a Member State or a zone if:

(a) vaccination against AD has been prohibited for kept porcine animals for the previous 12 months;

(b) surveillance has been carried out to demonstrate that no establishment in the respective Member State or zone has had any clinical, virological or serological evidence of infection with ADV for at least the previous 24 months; and

(c) in case, infection with ADV is known to be established in wild porcine animals, measures have been implemented to prevent any transmission of ADV from wild to kept porcine animals.

SECTION 2
MAINTENANCE OF THE STATUS

1. The status free from infection with ADV as regards kept porcine animals of a Member State or a zone may only be maintained if:

   (a) the requirements defined in points (a) and (c) of Section 1 continue to be fulfilled; and

   (b) surveillance is carried out annually based on random sampling to allow at least for the detection, with a 95 % level of confidence, of establishments infected with ADV at a target prevalence rate of 0.2 %. The number of blood or meat juice samples to be taken from the porcine animals kept in an establishment must allow at least for the detection, with a 95 % level of confidence, of seropositive animals at a target prevalence rate of 20 %.

2. By way of derogation from point 1, the status free from infection with ADV in the porcine population of a Member State or zone may be maintained in the event of an outbreak, if:

   (a) all the porcine animals in the affected establishments have been removed;

   (b) an epidemiological enquiry and investigations including clinical examination and serological or virological testing has been carried out by the competent authority:

      (i) in all establishments keeping porcine animals that have been directly or indirectly in contact with the infected establishment to rule out infection; and

      (ii) in all establishments keeping porcine animals located within at least a 2-kilometre radius of an infected establishment, to demonstrate that these establishments are not infected. The number of blood or meat juice samples to be taken from porcine animals kept in these establishments must allow at least for the detection, with a 95 % level of confidence, of seropositive animals at a target prevalence rate of 10 %; or

      (iii) in case a DIVA vaccination has been used, serological testing for antibodies against ADV-gE has been carried out on two occasions at an interval of 2 months in establishments keeping porcine animals located within the vaccinated radius from the infected establishment to demonstrate the absence of infection;

   (c) the result of the investigation according to point (b) has demonstrated that only a limited number of establishments were involved in the outbreak;

(d) the relevant control measures as referred to in Article 24 have been immediately implemented in each establishment infected with ADV, including where necessary vaccination with DIVA vaccines.

3. By way of derogation from point (a) of Section 1, the use of DIVA vaccination may be authorised by the competent authority in the event of an outbreak referred to in point 2, if:

(a) its use is limited to control this outbreak as deemed necessary by the competent authority;

(b) the porcine animals are DIVA vaccinated under the supervision of the competent authority and the use of DIVA vaccines is documented for each animal;

(c) the DIVA vaccinated porcine animals are only moved directly to a slaughterhouse or to an establishment in another Member State or zone where no vaccination ban is in place.

4. By way of derogation from point 1(b), surveillance may be carried out to demonstrate annually the absence of ADV infection taking into account the systems of production and the risk factors identified, provided no outbreaks have been detected for 2 consecutive years following the granting of the status free from infection with ADV in this Member state or zone.

Part VI
Bovine viral diarrhoea

Chapter 1
Establishment free from bovine viral diarrhoea

SECTION 1
GRANTING OF THE STATUS

1. The status free from bovine viral diarrhoea (BVD) may only be granted to an establishment keeping bovine animals if:

   (a) during the past 18 months there has been no confirmed case of BVD in a bovine animal kept in the establishment;

   (b) the bovine animals kept in the establishment have been subjected to at least one of the following testing regimes taking into account possible previous vaccinations:

      (i) tests for the detection of BVD virus (BVDV) antigen or genome have been carried out, with negative results, on samples of all bovine animals.

      At least from all calves born in the previous 12 months, the samples must have been taken after or at the same time as official identification, but not later than 20 days postpartum. The dams of those calves with negative test results do not need to be tested;

      (ii) serological tests for the detection of antibodies against BVDV have been carried out, with negative results, on samples taken over a period of not less than 12 months on at least three occasions at intervals of not less than 4 months from bovine animals which have been kept in the establishment for at least 3 months prior to testing.

      The number of animals tested must allow at least for the detection, with a 95 % level of confidence, of seropositive animals at a target prevalence rate of 50 % and must be at least five animals or all the animals if there are fewer than five animals kept.

      In case the bovine animals of the establishment are kept in separate groups without direct contact with each other, a respective number of animals of each group must be tested;

      (iii) a combination of the testing regimes set out in points (i) and (ii) has been applied over a period of not less than 12 months.

      The capacity of the combined testing regime to detect the disease must be equivalent to that of the testing regimes referred to in points (i) and (ii);

   (c) since the beginning of the sampling referred to in point 1 (b), all bovine animals introduced into the establishment:

      (i) originate from establishments free from BVD located in a Member State or zone free from BVD; or

(ii) originate from establishments free from BVD, where

– serological tests referred to in point 1(c) (ii) or (iii) of Section 2 of Chapter 1 have been carried out, with negative results, within the past 4 months; or

– prior to their dispatch, they have been tested individually to exclude BVDV transmission into the establishment of destination taking into account the testing history and, if relevant, the animal's stage of gestation; or

(iii) have tested negative in a test for BVDV antigen or genome, and

– have been subjected to quarantine for a period of at least 21 days prior to their dispatch and, in case of pregnant dams, have tested negative for antibodies against BVDV on samples taken after not less than 21 days of quarantine; or

– have tested positive for antibodies against BVDV either prior to their dispatch or, in case of pregnant dams, before insemination preceding the current gestation;

(d) since the beginning of the sampling referred to in point 1(b) all germinal products of bovine animals introduced into the establishment originate from:

(i) establishments free from BVD; or

(ii) approved germinal product establishments.

2. By way of derogation from point 1, the status free from BVD may be granted to an establishment if:

(a) all bovine animals originate from establishments free from BVD located in a Member State or zone free from BVD or in a Member State or zone covered by an approved eradication programme and fulfil the requirements laid down in point 1(c), if relevant; or

(b) all bovine animals originate from establishments free from BVD, are not intended for breeding and the status free from BVD of the establishment is maintained in accordance with point 2 of Section 2.

SECTION 2
MAINTENANCE OF THE STATUS

1. The status free from BVD of an establishment keeping bovine animals may only be maintained if:

(a) the requirements laid down in point (a), (c) and (d) of point 1 of Section 1 continue to be fulfilled;

(b) no bovine animal has been vaccinated against BVD since the status free from BVD was granted to the establishment;

(c) at least one of the following testing regimes is carried out with negative results:

(i) each new-born calf is tested negative for BVDV antigen or genome on a sample taken after or at the same time as official identification, but not later than 20 days postpartum;

(ii) serological tests for the detection of antibodies against BVDV are carried out at least annually on samples taken from bovine animals that have been kept in the establishment for at least 3 months prior to testing.

The number of animals tested must allow at least for the detection, with a 95 % level of confidence, of seropositive animals at a target prevalence rate of 50 % and must be at least five animals or all the animals if there are fewer than five animals kept;

In case the bovine animals of the establishment are kept in separate groups without direct contact with each other, a respective number of animals of each group must be tested;

(iii) a combination of the testing regimes laid down in points (i) and (ii) is applied.

The capacity of the combined testing regime to detect the disease must be equivalent to that of the testing regimes referred to in points (i) and (ii);

(iv) if the establishment is located in a Member State or zone free from BVD, tests are carried out on samples taken in accordance with point 1(b) of Section 2 of Chapter 2 or point 3 of Section 2 of Chapter 2, if relevant;

(d) only bovine animals that have not been vaccinated against BVD are introduced into the establishment if it is located in a Member State or zone free from BVD.

2. By way of derogation from point 1, the status free from BVD of an establishment keeping bovine animals referred to in point 2(b) of Section 1 may be maintained without testing the bovine animals in accordance with point 1(c) if:

(a) the requirements laid down in point 2(b) of Section 1 continue to be fulfilled;

(b) they are not used for breeding;

(c) they have no contact with animals that are intended or used for breeding and are moved from this establishment to a slaughterhouse,

(i) directly, or;

(ii) via an assembly operation, which is carried out in the same Member State or zone, and where only animals that comply with the requirements laid down in points (b) and (c) and originate from establishments that comply with the requirement laid down in point (a) are assembled.

SECTION 3
SUSPENSION AND RESTORING OF THE STATUS

1. The status free from BVD of an establishment keeping bovine animals must be suspended if:

(a) one or more of the requirements laid down in Section 2 are not fulfilled;

(b) a case of BVD is suspected in a bovine animal kept in the establishment.

2. The status free from BVD may only be restored if:

(a) the requirements laid down in points (c) and (e) of point 1 of Section 1 and points (b), (c), (d) of point 1 and, if relevant, point 2 of Section 2 are fulfilled.

(b) the results of further investigations substantiate absence of BVD and the status of all suspected cases has been determined.

SECTION 4
WITHDRAWAL AND REGAINING OF THE STATUS

1. The status free from BVD of an establishment keeping bovine animals must be withdrawn if:

   (a) one or more of the requirements laid down in Section 2 are not fulfilled after the maximum period of time referred to in point (b) of Article 20(3) has lapsed since the status was suspended;

   (b) a case of BVD has been confirmed in a bovine animal kept in the establishment.

2. If the status free from BVD has been withdrawn in accordance with point 1(a), it may only be regained if the requirements laid down in points (c) and (e) of point 1 of Section 1 and points (b), (c) and (d) of point 1 and, if relevant, point 2 of Section 2 are fulfilled.

3. If the status free from BVD has been withdrawn in accordance with point 1(b), it may only be regained if:

   (a) all animals tested positive for BVDV have been removed;

   (b) the status in relation to infection with BVDV of each bovine animal kept in the establishment has been determined;

   (c) all calves that might have been infected in utero with BVDV were born and kept in isolation until they tested negative for BVDV antigen or genome.

Chapter 2
Member State or zone free from bovine viral diarrhoea

SECTION 1
GRANTING OF THE STATUS

The status free from BVD as regards kept bovine animals may only be granted to a Member State or a zone if:

(a) vaccination against BVD has been prohibited for kept bovine animals;

(b) no case of BVD has been confirmed in a kept bovine animal for at least the previous 18 months; and

(c) at least 99.8 % of the establishments representing at least 99.9 % of the bovine population are free from BVD.

SECTION 2
MAINTENANCE OF THE STATUS

1. The status free from BVD as regards kept bovine animals of a Member State or a zone may only be maintained if:

(a) the requirements laid down in point (a) and (c) of Section 1 continue to be fulfilled; and

(b) surveillance is carried out annually that must allow at least for the detection, with a 95 % level of confidence, of establishments infected with BVDV at a target prevalence rate of 0.2 % of the establishments or of BVDV infected bovine animals with a target prevalence rate of 0.1 % of the bovine population.

2. By way of derogation from point 1(a), the use of vaccination may be authorised by the competent authority in the event of an outbreak, if:

(a) the results of the epidemiological enquiry and investigations according to Article 25 have demonstrated that only a limited number of establishments were involved in the outbreak;

(b) only a limited number of bovine animals deemed necessary by the competent authority to control this outbreak are vaccinated under the supervision of the competent authority and the use of vaccination is documented for each animal.

3. By way of derogation from point 1(b), surveillance may be carried out to demonstrate annually the absence of BVD taking into account the systems of production and the risk factors identified, provided no outbreaks have been detected for 5 consecutive years following the granting of the status free from BVD in this Member state or zone.

ANNEX V

DISEASE SPECIFIC REQUIREMENTS FOR THE GRANTING AND MAINTENANCE OF THE DISEASE-FREE STATUS AT THE LEVEL OF MEMBER STATES OR ZONES

Part I
Infection with rabies virus

Chapter 1
Technical requirements for the vaccination against rabies

SECTION 1
VACCINATION OF KEPT ANIMALS

1. For the purpose of eradication programmes for infection with rabies virus (RABV), anti-rabies vaccination must only be carried out on pet animals that are identified and must fulfil the requirements laid down in Annex III to Regulation (EU) No 576/2013 of the European Parliament and of the Council[1].

2. For the purpose of eradication programmes for infection with RABV, anti-rabies vaccination of kept animals, other than those referred to in the first paragraph, must be risk-based and carried out with the purpose of protecting humans from being exposed to rabies virus, using vaccines that meet the requirements laid down in points (1)(a) and (1)(b)of Annex III to Regulation (EU) No 576/2013 of the European Parliament and of the Council.

SECTION 2
VACCINATION OF WILD ANIMALS

1. For the purpose of eradication programmes for infection with RABV the oral vaccination against infection with RABV of wildlife must:

   (a) be organised and implemented as regular planned or emergency campaigns taking into account the risk assessment provided in point (a) of Article 32(2);

   (b) be subjected to an adequate vaccine distribution in terms of timing and coverage of the vaccination area, taking into account the biology of the targeted animal population, the epidemiological situation and the topography of the area;

   (c) be subjected, with the support of geographical information systems, to assessment of the correct geographical distribution of vaccine baits with a frequency that allows, if necessary, the adoption of corrective measures; and

[1] Regulation (EU) No 576/2013 of the European Parliament and of the Council of 12 June 2013 on the non-commercial movement of pet animals and repealing Regulation (EC) N0 998/2003 (OJ L 178, 28.6.2013, p.1).

(d) be subjected to monitoring of vaccination effectiveness, that may include the detection of the presence of biomarker and serological testing in dead animals from the targeted animal population for the vaccination.

2. For the purpose of eradication programmes for infection with RABV the vaccination against infection with RABV of stray dog populations must:

(a) be organised and implemented, if necessary, as part of control and management measures of stray dog populations, taking into account the risk assessment provided for in point (a) of Article 32(2);

(b) comply with the requirements of Section 1.

Chapter 2
Member State or zone free from infection with rabies virus

SECTION 1
GRANTING OF THE STATUS

1. The status free from infection with RABV may only be granted to a Member State or a zone if:

(a) surveillance has been implemented in accordance with the requirements laid down in Article 3(1) at least for the past 24 months; and

(b) no case of infection with RABV has been confirmed during the past 24 months in the targeted animal population.

2. By way of derogation from point 1(b), if a case of infection with RABV has been confirmed, the status may be granted if the infection of the case did not occur in the Member State or in the zone; and

(a) the case has been officially confirmed and no epidemiological link may have occurred and resulted in any additional case, which includes detection of the case at a border control post, or in a quarantine establishment or the quarantine facilities of a confined establishment; or

(b) epidemiological link may have occurred and no additional case was detected by increased surveillance and epidemiological enquiry and investigations during the 6 months following the death of the case.

SECTION 2
MAINTENANCE OF THE STATUS

The status free from infection with RABV of a Member State or a zone may only be maintained if:

(a) surveillance is implemented in accordance with the requirements laid down in Article 3(1) with the objective of an early detection of the disease; and

(b) no case of infection with RABV has been confirmed in the targeted animal population or a case occurred and the conditions laid down in point 2 of Section 1 were complied with.

Part II
Infection with bluetongue virus (serotypes 1-24)

Chapter 1
Minimum requirements for the surveillance

SECTION 1
SURVEILLANCE FOR THE DETECTION OF SEROTYPES OF BLUETONGUE VIRUS NOT REPORTED IN THE PREVIOUS 2 YEARS

1. The surveillance of infection with bluetongue virus (serotypes 1-24) (infection with BTV) to ensure early detection of introduction or recurrence of infection with any of the serotypes 1-24 of BTV that were not reported during the previous 2 years must include:

   (a) general surveillance requirements as provided for in point (a) of Article 3(1);

   (b) active surveillance as provided for in Section 4.

2. The design of the surveillance provided for in point 1 must address:

   (a) the risk of infection with limited clinical manifestations;

   (b) the risk of introduction of BTV serotypes associated with the circulation of any of the serotypes 1-24 of BTV in the vicinity; and

   (c) any other identified relevant risk factor for introduction of any of the serotypes 1-24 of BTV not reported in the previous 2 years.

3. The surveillance in an area(s) adjacent to any infected Member State, zone or third country must be increased in an area of up to 150 km from the limit with the Member State, zone, or third country. The demarcation of the area of increased surveillance may be adapted to relevant ecological or geographical features likely to facilitate or interrupt the transmission of BTV or adapted due to the implementation of disease control measures that supports the choice between a greater or lesser distance.

4. The surveillance provided for in point 1(b) and point 3 must have the capacity at least to detect, with a 95 % level of confidence, the infection in the targeted animal population at a target prevalence rate of 5 %, unless otherwise specified in Section 2 of Chapter 4.

SECTION 2
SURVEILLANCE TO DETERMINE THE EXTENT OF INFECTION WITH BTV

1. The surveillance of infection with BTV to ensure the timely demarcation of the spread of the infection when one or more serotypes of BTV is present and, if necessary, to monitor the prevalence rate must include:

   (a) general surveillance requirements as provided for in point (a) of Article 3(1); and

   (b) active surveillance as provided for in Section 4.

2. The design of the surveillance provided for in point 1 must take into account: all available information on the epidemiology of the disease and biology of the vector that prevail on the territory.

3. The target prevalence rate of the surveillance provided for in point 1 must be adapted to the epidemiological situation, taking into the main risk factors such as the targeted animal population and the vector population.

SECTION 3
SURVEILLANCE TO DEMONSTRATE ABSENCE OF INFECTION WITH BTV

1. The surveillance of infection with BTV to demonstrate the absence of infection with any of the serotypes 1-24 that has been previously detected in the territory must include:

   (a) general surveillance requirements as provided for in point (a) of Article 3(1); and

   (b) active surveillance as provided for in Section 4.

2. The design of the surveillance provided for in point 1 must address:

   (a) the risk of infection with limited clinical manifestations;

   (b) all available information on the epidemiology of the disease and biology of the vector that prevail on the territory; and

   (c) any specific risk of persistence of the infection identified.

3. The surveillance provided for in point 1(b) must have the capacity at least to detect, with a 95 % level of confidence, the infection in the targeted animal population at a target prevalence rate of 1 %.

SECTION 4
REQUIREMENTS FOR THE ACTIVE SURVEILLANCE OF INFECTION WITH BTV

1. The geographical units referred to in point (a) of Article 40(1) must be based on a grid of 45 km by 45 km and can be adapted to:

   (a) the epidemiological situation, how fast the infection is spreading and the shape and size of the zones covered by the eradication programme in the event of confirmation of the infection; and

   (b) the zones in accordance with point (b) of Article 13(2).

2. Active surveillance must be based on one or a combination of the following activities:

   (a) monitoring of sentinel animals using serological or virological testing; and

   (b) structured prevalence surveys, based on a random or risk-based sampling strategy using serological or virological testing.

3. The frequency of the sampling must:

   (a) at least be annual, in the period of the year when infection or seroconversion is most likely to be detected; and

(b) be monthly during the vector activity season where regular information is needed due to the risk of the infection spreading.

4. The animals sampled must:

(a) not be vaccinated against the serotype(s) of BTV targeted for surveillance;

(b) no longer be covered by maternal immunity in case their mother was vaccinated or infected;

(c) be resident for a sufficient time in the relevant geographical unit and not have been protected from exposure to the vector;

(d) be representative for the geographical distribution of the targeted animal population in the relevant geographical unit; and

(e) be initially seronegative when surveillance is based on serological testing of sentinels.

5. The sample size in each geographical unit must be calculated in accordance with the target prevalence rate based on the objectives assigned in Sections 1-3.

6. When surveillance must be adapted as provided for in point (c) of Article 43(2), it must at least include a survey:

(a) on the introduced animals that:

(i) must be based on the sampling and testing of all introduced animals;

(ii) must take place as soon as possible after their introduction; or

(b) on the targeted animal population the most at risk due to the possible circulation of the virus that:

(i) must have a capacity at least to detect, with a 95 % level of confidence, infection with BTV at a target prevalence rate of 5 %;

(ii) must either:

– not take place before 21 days has elapsed after the introduction of animals if it is a one-shot survey; or

– must be conducted with a frequency adapted to the frequency of the movements of the animals that may jeopardize the health status.

This survey is not required if the frequency of the sampling is carried out in accordance with point 3(b).

SECTION 5
ENTOMOLOGICAL SURVEILLANCE

1. Entomological surveillance must consist of at least an active annual programme of vector catching by means of permanently sited aspiration traps intended to determine the population dynamics of the vector and, where relevant, the vector-free period.

2. Aspiration traps equipped with ultraviolet light must be used in accordance with pre-established protocols; the traps must be operated throughout the night and operate at least:

(a) one night per week during the month before the expected beginning and during the month before the expected end of the vector-free period; and

(b) one night per month during the vector-free period.

On the basis of the evidence obtained in the first 3 years of operating the aspiration traps, the frequency of operation of those traps may be adjusted.

3. At least one aspiration trap must be placed in each geographical unit referred to in point (a) of Article 40(1) throughout the seasonally BTV-free zone. A proportion of the midges collected in the aspiration traps must be sent to a specialised laboratory capable of counting and identifying the suspected vector species or complexes.

4. When entomological surveillance is organised in the context of determination of a vector-free period, a maximum threshold of *Culicoides* species must be defined for the interpretation of the results. In the absence of sound evidence supporting the determination of the maximum threshold, total absence of *Culicoides imicola* specimens and less than five parous *Culicoides* per trap must be used as maximum threshold.

Chapter 2
Movement of animals and germinal products

SECTION 1
MOVEMENT OF ANIMALS

1. The animals originate from a Member State or a zone free from infection with BTV and have not been vaccinated with a live vaccine against infection with BTV in the last 60 days before the date of movement.

2. The animals originate from a Member State or a zone covered by the eradication programme and at least one of the following requirements is complied with:

   (a) the animals have been kept in a seasonally BTV-free Member State or zone established in accordance with paragraph 3 of Article 40:

      (i) for at least 60 days prior to the date of movement;

      (ii) for at least 28 days prior to the date of movement and have been subjected to a serological test, with negative results, carried out on samples collected at least 28 days following the entry date of the animal into the seasonally BTV-free Member State or zone; or

      (iii) for at least 14 days prior to the date of movement and have been subjected to a PCR test, with negative results, carried out on samples collected at least 14 days following the entry date of the animal into the seasonally BTV-free Member State or zone;

   (b) the animals have been protected against attacks by the vectors during transportation to the place of destination and they have been kept protected against attacks by vectors in a vector protected establishment:

      (i) for at least 60 days prior to the date of movement; or

      (ii) for at least 28 days prior to the date of movement and have been subjected to a serological test, with negative results, carried out on samples collected at least 28 days following the date of the commencement of the period of protection against attacks by vectors; or

(iii) for at least 14 days prior to the date of movement and have been subjected to a PCR test, with negative results, carried out on samples collected at least 14 days following the date of commencement of the period of protection against attacks by vectors;

(c) the animals have been vaccinated against all serotypes 1-24 of BTV reported during the past 2 years in that Member State or zone, the animals are within the immunity period guaranteed in the specifications of the vaccine and meet at least one of the following requirements:

(i) they have been vaccinated more than 60 days before the date of movement; or

(ii) they have been vaccinated with an inactivated vaccine and subjected to a PCR test, with negative results on samples collected at least 14 days after the onset of the immunity set in the specifications of the vaccine.

(d) the animals have been subjected with positive results to a serological test able to detect specific antibodies against all serotypes 1-24 of BTV reported during the past 2 years in that Member State or zone and:

(i) the serological test have been carried out on samples collected at least 60 days before the date of movement; or

(ii) the serological test have been carried out on samples collected at least 30 days before the date of the movement and the animals have been subjected to a PCR test, with negative results, carried out on samples collected not earlier than 14 days before the date of movement.

3. The animals originate from a Member State or a zone neither BTV-free nor covered by an eradication programme for infection with BTV and:

(a) they comply with point 2(b); or

(b) the animals have been kept at least for the last 60 days prior to departure either in an area of at least 150 km radius from the establishment where they are kept, or in a Member State, where surveillance in compliance with the requirements laid down in Sections 1 and 2 of Chapter 1 have been carried out at least for the last 60 days prior to departure and:

(i) they have been vaccinated in accordance with point 2(c) against all serotypes 1-24 of BTV reported during the past 2 years in an area of at least 150 km radius centred on the place where the animals were kept; or

(ii) they have been immunised in accordance with point 2(d) against all serotypes 1-24 of BTV reported during the past 2 years in an area of at least 150 km radius centred on the place where the animals were kept.

4. The animals originate from a Member State or a zone not BTV-free, are destined for immediate slaughter and the following requirements apply:

(a) no case of infection with BTV has been reported in the establishment of origin for a period of at least 30 days prior to the date of movement;

(b) the animals are transported directly from the Member State or zone of origin to the slaughterhouse of destination where they are slaughtered within 24 hours of arrival;

(c) the operator of the establishment of origin have informed the operator of the slaughterhouse of destination of the movement at least 48 hours prior to the loading of the animals.

5. The animals originate from a Member State or a zone neither BTV-free nor covered by an eradication programme for infection with BTV and the animals comply with the requirements set out in point 2(a).

6. The animals originate from a Member State or a zone not BTV-free and:

(a) they have been protected from vector attacks by insecticides or repellents for at least 14 days prior to the date of movement; and

(b) they have been subjected during that period to a PCR test, with negative results, carried out on samples collected at least 14 days following the date of protection from vector attacks.

7. The animals comply with specific animal health requirements defined by the competent authority to ensure they have sufficient immunological protection prior to departure.

8. The animals comply with any of the requirements provided for in points 2, 3 5, 6 or 7 only for the serotypes of BTV reported for the past 2 years in the Member State or zone of origin and not in the Member State or zone of destination during the same period.

SECTION 2
MOVEMENT OF GERMINAL PRODUCTS

1. The donor animals have been kept at least for a period of 60 days prior to and during the collection of germinal products in a Member State or a zone free from infection with BTV.

2. The germinal products originate from a Member State or a zone covered by the eradication programme for infection with BTV and at least one of the requirements set out in point (a) for semen, point (b) for in vivo derived embryos of bovine animals or point (c) for embryos other than in vivo derived embryos of bovine animals and oocytes is complied with:

(a) semen have been obtained from donor animals that comply with at least one of the following requirements:

(i) they have been protected against attacks by vectors in a vector protected establishment for a period of at least 60 days before commencement of collection and during collection of the semen;

(ii) they have been kept in a seasonally BTV-free Member State or zone for a period of at least 60 days before commencement of collection and during collection of the semen;

(iii) they have been subjected to a serological test, with negative results, on samples collected between 28 and 60 days from the date of each collection of the semen;

(iv) they have been subjected, with negative results, to a direct diagnostic method carried out on samples collected:

– at commencement and final collection of the semen to be consigned; and

– during the period of semen collection: at least every 7 days in the case of a virus isolation test, or at least every 28 days, in the case of a PCR test;

(b) in vivo derived embryos of bovine animals have been obtained from donor animals that do not show any clinical signs of infection with BTV on the day of collection and are collected, processed and stored in accordance with Part 2 of Annex III of Commission Delegated Regulation (EU) 20XX/ … [SANTE/7073/2018, C(2019)4055][2];

(c) embryos other than in vivo derived embryos of bovine animals and oocytes have been obtained from donor animals that comply with at least one of the following requirements:

(i) they have been protected against attacks by vectors in a vector protected establishment for at least 60 days before commencement of collection and during collection of the embryos/oocytes;

(ii) they have been subjected to a serological test, with negative results, on samples collected between 28 and 60 days from the date of each collection of the embryos/oocytes;

(iii) they have been subjected to a PCR test, with negative results, on samples collected on the day of collection of the embryos/oocytes;

(iv) they have been kept in a seasonally BTV-free Member State or zone for a period of at least 60 days before collection of the embryos/oocytes.

3. The germinal products originate from a Member State or a zone neither BTV-free nor covered by an eradication programme for infection with BTV and comply with the requirements set out either in point 2(a)(i), 2(a)(iii), 2(a)(iv), 2(b), 2(c)(i), 2(c)(ii) or 2(c)(iii).

4. The germinal products originate from a Member State or a zone neither BTV-free nor covered by an eradication programme for infection with BTV and must comply with either point 2(a)(ii) or 2(c)(iv).

Chapter 3
Vector protected establishment

The status of vector protected establishment may only be granted to an establishment if:

(a) it has appropriate physical barriers at entry and exit points;

(b) openings must be vector screened with mesh of appropriate gauge which must be impregnated regularly with an approved insecticide according to the manufacturers' instructions;

[2] Commission Delegated Regulation (EU) 20XX/ … of ..,supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards the approval of germinal product establishments and the traceability and animal health requirements for movements within the Union of germinal products of certain kept terrestrial animals [SANTE/7073/2018, C(2019)4055]

(c) vector surveillance and control must be carried out within and around the vector protected establishment;

(d) measures must be taken to limit or eliminate breeding sites for vectors in the vicinity of the vector protected establishment; and

(e) standard operating procedures must be in place, including descriptions of back-up and alarm systems, for operation of the vector protected establishment and transport of animals to the place of loading.

Chapter 4
Member State or zone free from infection with BTV

SECTION 1
GRANTING OF THE STATUS

1. The status free from infection with BTV may only be granted to a Member State or to a zone, where BTV has never been reported, if:

   (a) surveillance in accordance with Section 1 of Chapter 1 has been conducted at least for the past 24 months; and

   (b) no case of infection with BTV has been confirmed during the past 24 months in the targeted animal population.

2. The status free from infection with BTV may only be granted to a Member State or to a zone where BTV has already been reported if:

   (a) surveillance in accordance with Section 3 of Chapter 1 has been conducted at least for the past 24 months; and

   (b) no case of infection with BTV has been confirmed during the past 24 months in the targeted animal population.

SECTION 2
MAINTENANCE OF THE STATUS

1. The status free from infection with BTV may only be maintained if:

   (a) the requirements laid down in point 1 of Section 1 are complied with; and

   (b) animals and germinal products from the targeted animal population are only moved into or through the Member State or zone when the requirements laid down in Articles 43 and 45 are complied with.

2. The intensity and frequency of the surveillance referred to in point 1 of Section 1 must be duly adapted to:

   (a) the health status of neighbouring Member States, zones or third countries in accordance with point 3 of Section 4 of Chapter 1;

   (b) the introduction of animals from the targeted animal population that may have jeopardized the health status of the Member State or zone, in accordance with point 6 of Section 4 of Chapter 1.

3. If no circulation of the infection has been detected for 2 consecutive years following granting of the status free from infection with BTV of a Member State or of a zone, surveillance must be based on:

(a) random annual surveillance at least to detect, with a 95 % level of confidence, the infection with BTV at a target prevalence rate of 20 %; or

(b) risk-based annual surveillance to detect infection with BTV carried out taking into account the systems of production and the risk factors identified.

Chapter 5
Seasonally BTV-free Member State or zone

1. The seasonally BTV-free status may only be established in a Member State or zone thereof if:

(a) the beginning and the end of the vector-free period and therefore of the seasonally BTV-free period has been demonstrated based on entomological surveillance in accordance with Section 5 of Chapter 1; and

(b) the cessation of the transmission of BTV has been demonstrated by:

(i) the implementation of surveillance in accordance with Section 2 of Chapter 1 at least for the past 12 months including one full vector activity season; and

(ii) the absence of new cases of infection with any of the serotypes 1-24 of BTV since the end of the vector activity season.

2. By way of derogation from point 1(a), if the seasonally BTV-free period has been successfully demonstrated for a period of 3 consecutive years, additional criteria such as temperature may replace entomological surveillance to substantiate the beginning and the end of the seasonally BTV-free period on the basis of scientific evidence.

3. The seasonally BTV-free Member State or zone must immediately stop when there is evidence of the end of the vector-free period or of circulation of the virus.

Part III
Infestation with *Varroa* spp.

SECTION 1
GRANTING OF THE STATUS TO A MEMBER STATE OR ZONE AS FREE FROM INFESTATION WITH *VARROA* SPP.

The status free from infestation with *Varroa* spp. may only be granted to the relevant honeybee population of a Member State or of a zone if:

(a) a risk assessment has been conducted, identifying all potential factors for *Varroa* spp. occurrence and its potential presence in the past;

(b) an ongoing awareness programme has been in place for at least one year to encourage reporting of all cases suggestive of *Varroa* spp.;

(c) there has been no confirmed case of infestation with *Varroa* spp. either in kept or in wild honeybee colonies;

(d) for at least one year, an annual surveillance has demonstrated the absence of infestations with *Varroa* spp. on a representative sample of kept honeybees of the Member State or zone thereof that allows at least for the detection, with a 95 % level of confidence, of the infestation with *Varroa* spp. at a target prevalence rate of 1 % of the apiaries and at a within-apiary target prevalence rate of 5 % of the beehives;

(e) in the presence of a wild self-sustaining population of the species of the genus *Apis* there has been in place for at least one year an ongoing surveillance programme in the wild population which demonstrates no evidence of infestation with *Varroa* spp.; and

(f) during the whole duration of the surveillance referred to in point (d) the competent authority makes appropriate arrangements for the survey and further handling of honeybees in any stage of their lifecycle, including honeybee brood, which are moved into that Member State or into that zone to prevent the infestation of its population from introduced honeybees of lesser health status.

SECTION 2
MAINTENANCE OF THE STATUS OF A MEMBER STATE OR ZONE FREE FROM INFESTATION WITH *VARROA* SPP.

The status free from infestation with *Varroa* spp. granted to the relevant honeybee population of a Member State or of a zone may only be maintained if:

(a) the competent authority maintains a surveillance that:

  (i) demonstrates the absence of infestations with *Varroa* spp. annually on a representative sample of kept honeybees of the free area;

  (ii) enables the early detection of infestation with *Varroa* spp. in apiaries and beehives;

  (iii) takes into consideration specifically target areas with higher likelihood of introduction of or infestation with *Varroa* spp., based on a risk assessment;

(b) all the suspected cases have been investigated and no case of infestation with *Varroa* spp. has been confirmed, either in kept or in wild honeybee colonies;

(c) either there is no wild self-sustaining population of the species of the genus Apis or there is an ongoing surveillance programme in the wild population which demonstrates no evidence of infestation with *Varroa* spp.; and

(d) the honeybees in any stage of their lifecycle, including honeybee brood, are only moved into the free area when:

(i) they come from a Member State or zone thereof or from a third country or territory with disease-free status regarding infestation with *Varroa* spp.; and

(ii) they are protected from infestation with *Varroa* spp. during transport.

Part IV
Status free from infection with Newcastle disease virus-without vaccination

SECTION 1
GRANTING OF STATUS FREE FROM INFECTION WITH NEWCASTLE DISEASE VIRUS WITHOUT VACCINATION

The status free from infection with Newcastle disease virus (NDV) status without vaccination in the population of poultry and captive birds of *Galliformes* species may only be granted to a Member State or to a zone if for at least the past 12 months:

(a) vaccination against infection with NDV in poultry and in captive birds of *Galliformes* species has been prohibited;

(b) no poultry and no captive birds of *Galliformes* species vaccinated against infection with NDV has been kept in establishments keeping poultry or captive birds of *Galliformes* species;

(c) general surveillance requirements have been carried out in accordance with point (a) of Article 3(1) for the early detection of infection with NDV;

(d) one of the following testing regime has applied:

  (i) all establishments keeping breeding poultry have been tested for the presence of antibodies against infection with NDV with negative results, on blood samples from at least 60 birds randomly chosen from each establishment and tested serologically by Haemagglutination inhibition (HI) test; or

  (ii) a survey has been conducted on a representative sample of establishments which has at least the capacity at least to detect, with a 95 % level of confidence, the infection at a target prevalence rate of 1 % in the poultry establishments and at a within-establishment prevalence rate of seropositive birds of 10 %; and

(e) no case of infection with NDV has been confirmed in poultry and captive birds of *Galliformes* species.

SECTION 2
MAINTENANCE OF THE STATUS

1. The status free from infection with NDV without vaccination granted to a Member State or to a zone may only be maintained if the requirements in points (a) to (e) of Section 1 continue to be fulfilled.

2. By way of derogation from paragraph 1, the status free from infection with NDV without vaccination granted to a Member State or to a zone may be maintained in the event of the confirmation of a case of infection with NDV if:

  (a) the relevant disease control measures have been immediately implemented on each establishment with suspected or confirmed cases until the incident has been resolved;

(b) the competent authority has concluded that only a limited number of establishments, epidemiologically linked to the first detected outbreak, were infected; and

(c) during a period of 12 months, the disease control measures referred to in point (a) were not applied for a duration longer than three months.

3. The status free from infection with NDV without vaccination granted to a Member State or to a zone is not affected by the confirmation of the infection in another bird population, provided the competent authority has assessed, taking into account the implementation of all necessary measures to prevent transmission of infection with NDV to poultry and captive birds of *Galliformes* species, that the status was not jeopardized.

ANNEX VI

SPECIFIC REQUIREMENTS AS REGARDS DISEASES OF AQUATIC ANIMALS

Part I

RISK-BASED SURVEILLANCE

Chapter 1
Minimum requirements for risk-based surveillance in certain approved aquaculture establishments

1. General approach

1.1. Risk-based health surveillance which includes health visits and possible sampling is applied in certain approved aquaculture establishments and in certain approved groups of aquaculture establishments in a manner that is appropriate to the nature of the production and which has the objective of detecting:

(a) increased mortality;

(b) listed diseases;

(c) emerging diseases.

1.2. The frequency of such visits will depend on the risk the approved aquaculture establishment or approved group of aquaculture establishments poses in relation to contracting and spreading disease. This risk applies to listed diseases and to potential emerging diseases and will therefore include aquaculture establishments and groups of aquaculture establishments keeping listed species and in certain cases, aquaculture establishments and groups of aquaculture establishments keeping non-listed species. The competent authority must determine the risk posed by each approved aquaculture establishment or approved group of aquaculture establishments and rank them as high, medium or low risk.

Chapter 2 provides details of the risk factors to be taken into account during the risk ranking process. Such risk ranking will be repeated and updated if any of the risk factors outlined in points (a) to (l) indicate that the risk posed by the establishment has changed.

1.3. Chapter 3 sets out the minimum frequency of health visits which must be completed, based on whether the competent authority has designated an establishment to be high, medium or low risk.

1.4. Risk-based animal health surveillance in aquaculture establishments and groups of aquaculture establishments may be combined with health visits and sampling which are carried out:

(a) as part of compulsory or optional eradication programmes for one or more listed diseases; or

(b) to demonstrate and maintain disease free status for one or more listed diseases; or

(c) as part of a surveillance programme for one or more category C diseases.

Chapter 2

Risk ranking to be applied in certain approved aquaculture establishments

The risk ranking referred to in point 1.2 of Chapter 1 must as a minimum, take into account the risk factors referred to in points (a) and (b). Where relevant, points (c) to (l) will also be considered:

(a) possibility of the direct spread of pathogens via water;

(b) movements of aquaculture animals;

(c) type of production;

(d) species of aquaculture animals kept;

(e) biosecurity system, including staff competence and training;

(f) density of aquaculture establishments and processing establishments in the area around the establishment concerned;

(g) proximity of establishments with a lower health status than the establishment concerned;

(h) disease history of the establishment concerned and of other local establishments;

(i) presence of infected wild aquatic animals in the area around the establishment concerned;

(j) risk posed by human activities in the proximity of the establishment concerned for example angling, the presence of transport routes, ports at which ballast water is exchanged;

(k) access to the establishment concerned by predators which may cause disease spread;

(l) track record of the establishment as regards compliance with the requirements of the competent authority.

Chapter 3

Frequency of risk-based animal health visits

The frequency of risk-based health visits which must be carried out in certain approved establishments and approved groups of establishments depends upon the risk ranking referred to in Chapter 2 and shall be carried out as follows:

(a) at least once per year in high risk establishments;

(b) at least once every two years in medium risk establishments;

(c) at least once every three years in low risk establishments.

Part II
DISEASE- SPECIFIC REQUIREMENTS FOR DISEASE-FREE STATUS OF AQUATIC ANIMALS

Part II covers the disease-specific requirements for disease-free status as regards the following listed diseases:

Viral haemorrhagic septicaemia (VHS)	Chapter 1
Infectious haematopoietic necrosis (IHN)	Chapter 1
Infection with HPR-deleted infectious salmon anaemia virus	Chapter 2
Infection with *Marteilia refringens*	Chapter 3
Infection with *Bonamia exitiosa*	Chapter 4
Infection with *Bonamia ostreae*	Chapter 5
Infection with white spot syndrome virus (WSSV)	Chapter 6

Chapter 1
Eradication, disease-free status and diagnostic methods for viral haemorrhagic septicaemia (VHS) and infectious hematopoietic necrosis (IHN)

SECTION 1
GENERAL REQUIREMENTS FOR HEALTH VISITS AND SAMPLING

Health visits and sampling for the surveillance referred to in point (b)(ii) of Article 3(2) must comply with the following requirements:

(a) health visits and, where appropriate sampling, must be carried out during the period of the year when the water temperature is below 14 °C or when temperatures below 14 °C are not reached, samples must be taken at the lowest annual temperatures;

(b) when targeted surveillance in wild populations is required due to the small number of aquaculture establishments in an eradication programme, the number and geographical distribution of sampling points must be determined to obtain a reasonable coverage of the Member State, zone or compartment. The sampling points must be representative of the different ecosystems where wild populations of susceptible species are located;

(c) when establishments or wild populations are to be subject to health visits or sampled more than once per year, in accordance with Sections 2 to 4, the intervals between the health visits and between the collection of samples must be at least 4 months, or as long as possible, taking into account the temperature requirements provided for in point (a);

(d) all production units, such as ponds, tanks and net cages, must be examined for the presence of dead, weak or abnormally behaving fish. Particular attention must be

paid to the water outlet area where weak fish tend to accumulate because of the water current;

(e) fish of listed species to be collected as samples must be selected as follows:

(i) if rainbow trout are present, only fish of that species must be selected for sampling, except where other susceptible species are present which show typical signs of VHS or IHN; if rainbow trout are not present, the sample must be representative of all other susceptible species which are present;

(ii) if weak, abnormally behaving or freshly dead but not decomposed fish are present, such fish must be selected; if more than one water source is utilised for fish production, fish representing all water sources must be included in the sample;

(iii) the fish selected must include fish collected in such a way that all production units, such as net cages, tanks and ponds, of the establishment, as well as all year classes, are proportionally represented in the sample.

SECTION 2
GRANTING OF THE STATUS FREE FROM VHS OR FREE FROM IHN IN MEMBER STATES, ZONES AND COMPARTMENTS OF UNKNOWN HEALTH STATUS

The status free from VHS or free from IHN may only be granted to a Member State, a zone or a compartment with an unknown health status with regard to VHS or IHN if:

(a) all establishments and, when required, sampling points in wild populations selected in accordance with point (b) of Section 1, have been subject to one of the following scheme:

(i) model A — 2-year scheme

The establishments or sampling points must have been subject to health visits and sampled for a minimum period of 2 consecutive years as laid down in Table 1.A.

During that 2-year period, the testing of all samples using the diagnostic methods set out in point 2 of section 5 must have produced negative results for VHS or IHN, and any suspicion of VHS or IHN must have been ruled out in accordance with the sampling and diagnostic methods set out in point 3 of Section 5;

(ii) model B — 4-year scheme with reduced sample size

The establishments or sampling points must have been subject to health visits and sampled for a minimum period of 4 consecutive years as laid down in Table 1.B. During that 4-year period, the testing of all samples using the diagnostic methods set out in point 2 of Section 5 must have produced negative results for VHS or IHN and any suspicion of VHS or IHN must have been ruled out in accordance with the sampling and diagnostic methods set out in point 3 of Section 5;

(b) if VHS or IHN have been detected during the surveillance referred to in point (a); before starting a new 2-year or 4-year scheme, relevant establishments in the Member State, zone or compartment must:

(i) be subject to the minimum disease control measures laid down in Articles 58 to 65;

(ii) be repopulated with fish from an establishment in a Member State, zone or compartment with status free from VHS or status free from IHN or from an establishment in a Member State, zone or compartment covered by an eradication programme for VHS or IHN.

Table 1.A

Scheme for Member States, zones and compartments for the 2-year control period referred to in point (a)(i) which precedes the achievement of status free from VHS and status free from IHN

Type of establishment	**Number of health visits per year to each establishment**	**Number of samplings per year in each establishment**	**Number of fish in the sample**(1)	
			Number of growing fish	**Number of broodstock fish**(2)
(a) Establishments with broodstock	2	2	50 (first visit) 75 (second visit)	30 (first or second visit)
(b) Establishments with broodstock only	2	1	0	75 (first or second visit)
(c) Establishments without broodstock	2	2	75 (first AND second visit)	0
Maximum number of fish per pool: 10				

(1) In the case of coastal zones or coastal compartments, the samples must be collected no sooner than 3 weeks after the transfer of the fish from fresh to saltwater.

(2) Ovarian or seminal fluid of broodstock shall be collected at the time of maturation, in connection with stripping.

Table 1.B

Scheme for Member States, zones or compartments using a reduced sample size for the 4-year control period referred to in point (a)(ii) which precedes the achievement of status free from VHS and status free from IHN

Type of establishment	Number of health visits per year to each establishment	Number of samplings per year in each establishment	Number of fish in the sample([1])	
			Number of growing fish	Number of broodstock fish([2])
First 2 years				
(a) Establishments with broodstock	2	1	30 (second visit)	0
(b) Establishments with broodstock only	2	1	0	30 (first or second visit)
(c) Establishments without broodstock	2	1	30 (first or second visit)	0
Last 2 years				
(a) Establishments with broodstock	2	2	30 (first visit)	30 (second visit)
(b) Establishments with broodstock only	2	2		30 (first AND second visit)
(c) Establishments without broodstock	2	2	30 (first AND second visit)	
Maximum number of fish per pool: 10				

([1]) In the case of coastal zones or coastal compartments, the samples must be collected no sooner than 3 weeks after the transfer of the fish from fresh to saltwater.

([2]) Ovarian or seminal fluid of broodstock shall be collected at the time of maturation, in connection with stripping.

SECTION 3
GRANTING OF THE STATUS FREE FROM VHS OR FREE FROM IHN IN MEMBER STATES, ZONES AND COMPARTMENTS KNOWN TO BE INFECTED WITH EITHER VHS OR IHN

1. The status free from VHS or free from IHN may only be granted to a Member State, a zone or a compartment known to be infected with VHS or IHN, if all establishments keeping listed species within that Member State, zone or compartment have been subject to an eradication programme that complies with the following requirements:

   (a) the minimum control measures laid down in Articles 55 to 65 must have been effectively applied and a restricted zone of an appropriate size as provided for in point (c) of Article 58(1), where appropriate, divided into a protection zone and surveillance zone; must have been established in the vicinity of the establishment(s) declared infected with VHS or IHN, taking into account the requirements set out in point 2;

   (b) all establishments keeping listed species within the protection zone, or where a protection zone has not been established, the restricted zone, not infected with VHS or IHN must be subject to an investigation comprising at least the following elements:

      (i) the collection of samples for testing of 10 fish, when clinical signs or *post-mortem* lesions consistent with infection with VHS or IHN are observed or minimum 30 fish, when clinical signs or *post-mortem* lesions are not observed;

      (ii) in those establishments where the tests referred to in (i) have produced negative results; health visits must continue once per month during the period when the water temperature is below 14 °C, except when fish ponds, tanks, raceways or net cages are covered with ice, until the protection zone is withdrawn in accordance with point (c);

   (c) relevant establishments must be emptied in accordance with Article 62, cleaned and disinfected in accordance with Article 63 and fallowed in accordance with Article 64.

   The duration of the fallowing period referred to in point (a) of Article 64(2) must be at least 6 weeks. When all establishments infected within the same protection zone, or where a protection zone has not been established, the restricted zone, are emptied, at least 3 weeks of synchronised fallowing must be carried out.

   When fallowing of the infected establishments is carried out, the restricted zone or the protection zone, when it has been established, must be converted into a surveillance zone until the scheme set out in Section 2 is completed;

   (d) repopulation may only take place when all infected establishments have been emptied, cleaned, disinfected and fallowed in accordance with point (c);

   (e) all establishments other than those referred to in point (f) which keep listed species within the Member State, zone or compartment covered by the eradication programme and when surveillance in wild populations is required,

all sampling points selected in accordance with point (b) of Section 1, must subsequently be subject to the scheme laid down in Section 2;

(f) an individual establishment which keeps listed species and which has a health status which is independent of the health status of the surrounding waters is not required to comply with the scheme laid down in Section 2 following a disease outbreak, provided the establishment complies with the requirements set out in paragraph 3 of Article 80 and is repopulated with fish sourced from Member States, zones or compartments with status free from VHS or status free from IHN.

2. The restricted zone must have been defined on a case-by-case basis and:

(a) it must take into account factors influencing the risks for the spread of VHS or IHN to kept and wild fish, such as:

(i) the number, rate and distribution of the mortalities of fish on the establishment infected with VHS or IHN, or in other aquaculture establishments;

(ii) the distance to and density of neighbouring establishments;

(iii) the proximity to slaughterhouses;

(iv) contact establishments;

(v) species present at the establishments;

(vi) the farming practices applied in the infected establishments and the neighbouring establishments ;

(vii) the hydrodynamic conditions; and

(viii) other factors of epidemiological significance identified;

(b) the geographical demarcation in coastal areas must comply with the following minimum requirements:

(i) the protection zone must consist of an area included in a circle with a radius of at least one tidal excursion or at least 5 km, whichever is larger, centred on the establishment infected with VHS or IHN, or an equivalent area determined according to appropriate hydrodynamic or epidemiological data; and

(ii) the surveillance zone must consist of an area surrounding the protection zone, of overlapping tidal excursion zones; or an area surrounding the protection zone and included in a circle of radius 10km from the centre of the protection zone; or an equivalent area determined according to appropriate hydrodynamic or epidemiological data;

or

(iii) where separate protection and surveillance zones are not established, the restricted zone must consist of an area comprising both the protection zone and the surveillance zone;

(c) the geographical demarcation in inland areas must comprise the entire water catchment area in which the establishment infected with VHS or IHN is located. The competent authority may limit the extent of the restricted zone to

parts of the water catchment area, provided this limitation does not compromise the disease control measures with respect to VHS or IHN.

SECTION 4
MAINTENANCE OF STATUS FREE FROM VHS AND STATUS FREE FROM IHN

1. When targeted surveillance is required in order to maintain the status free from VHS or free from IHN of a Member State, a zone or a compartment, in accordance with Article 81, all establishments keeping listed species within the Member State, zone or compartment concerned must be subject to health visits and fish must be sampled in accordance with Table 1.C, taking into account the risk level of the establishment for the contraction of VHS or IHN.

2. When determining the frequency of health visits required to maintain the status free from VHS or the status free from IHN of compartments, where the health status regarding VHS or IHN is dependent on the health status of the aquatic animal populations in surrounding natural waters, the risk for the contraction of VHS or IHN must be regarded as high.

3. Disease-free status must only be maintained as long as all samples tested, using the diagnostic methods set out in point 2 of Section 5, have produced negative results for VHS or IHN and any suspicion of VHS or IHN has been ruled out in accordance with the diagnostic methods set out in point 3 of Section 5.

Table 1.C

Scheme for Member States, zones or compartments to maintain status free from VHS or status free from IHN

Risk level(1)	**Number of health visits per year to each establishment**	**Number of fish in the sample**(2,3)
High	1 every year	30
Medium	1 every 2 years	30
Low	1 every 3 years	30
Maximum number of fish per pool: 10		

(1) Risk level assigned to the establishment by the competent authority as set out in Chapter 2 of Part I other than in the case of dependent compartments where all establishments are deemed to be high risk.

(2) One sample to be taken during every health visit.

(3) In the case of coastal zones or coastal compartments, the samples must be collected no sooner than 3 weeks after the transfer of the fish from fresh to saltwater.

SECTION 5
DIAGNOSTIC AND SAMPLING METHODS

1. The organs or tissue material to be sampled and examined must be the spleen, the anterior kidney, and either heart or encephalon. When sampling broodstock, ovarian or seminal fluid may also be examined.

   In case of small fry, whole fish may be sampled.

Samples from a maximum of 10 fish may be pooled.

2. The diagnostic method for the granting or the maintenance status free from VHS or status free from IHN in accordance with Sections 2 to 4 must be:

   (a) virus isolation in cell culture with subsequent identification of the virus using ELISA, indirect fluorescent antibody test (IFAT), virus neutralisation test or virus genome detection; or

   (b) Reverse Transcription quantitative PCR (RT-qPCR) detection.

   The detailed procedures to carry out these diagnostic methods must be those approved by the EURL for fish diseases.

3. When a suspicion of VHS or IHN is required to be confirmed or ruled out in accordance with Article 55, the following health visit, sampling and testing procedures must comply with the following requirements:

   (a) the suspected establishment must be subject to at least one health visit and one sampling of 10 fish, when clinical signs or *post-mortem* lesions consistent with infection with VHS or IHN are observed or minimum 30 fish, when clinical signs or *post-mortem* lesions are not observed. Samples shall be tested using one or more of the diagnostic methods set out in points 2(a) and 2(b) in accordance with the detailed diagnostic methods and procedures approved by the EURL for fish diseases;

   (b) the presence of VHS must be considered as confirmed, if one or more of those diagnostic methods are positive for VHSV. The presence of IHN must be considered as confirmed, if one or more of those diagnostic methods are positive for IHNV. The confirmation of the first case of VHS or IHN in Member States, zones or compartments previously not infected must be based on conventional virus isolation in cell culture with subsequent immunochemical or molecular identification or with genome detection including confirmation by sequencing of the amplification (RT-PCR) product;

   (c) Suspicion of VHS or IHN may be ruled out, if cell cultivation or RT-qPCR tests reveal no further evidence of the presence of VHSV or IHNV.

Chapter 2

Eradication, disease-free status and diagnostic methods for infection with HPR-deleted infectious salmon anaemia virus (HPR-deleted ISAV)

SECTION 1

GENERAL REQUIREMENTS FOR HEALTH VISITS AND SAMPLING

Health visits and sampling for the surveillance referred to in point (b)(ii) of Article 3(2) must comply with the following requirements:

(a) when health visits and sampling of establishments must be carried out more than once per year in accordance with Sections 2 to 4, the intervals between the health visits or collection of samples shall be as long as possible;

(b) when targeted surveillance in wild populations is required due to the low number of aquaculture establishments in the eradication programme, the number and geographical distribution of sampling points must be determined to obtain a reasonable coverage of the Member State, zone or compartment;

(c) the sampling points must be representative of the different ecosystems where the wild populations of susceptible species are located;

(d) all production units, such as ponds, tanks and net cages, must be examined for the presence of dead, weak or abnormally behaving fish. Particular attention must be paid to the edge of cages or the water outlet area as relevant, where weak fish tend to accumulate because of the water current;

(e) fish of listed species to be collected as samples must be selected as follows:

(i) if Atlantic salmon are present, only fish of that species must be selected for sampling, except where other susceptible species are present which show typical signs of infection with HPR- deleted ISAV. If there are no Atlantic salmon in the establishment, the sample must be representative of all other susceptible species which are present;

(ii) if moribund or freshly dead, but not decomposed fish are present, such fish must be selected, in particular fish demonstrating anaemia, haemorrhages or other clinical signs suggesting circulatory disturbances; if more than one water source is utilised for fish production, fish representing all water sources must be included in the sample;

(iii) the fish selected must include fish collected in such a way that all production units, such as net cages, tanks and ponds, of the establishment as well as all year classes are proportionally represented in the sample.

SECTION 2

GRANTING OF THE STATUS FREE FROM INFECTION WITH HPR-DELETED ISAV IN MEMBER STATES, ZONES AND COMPARTMENTS OF UNKNOWN HEALTH STATUS

The status free from infection with HPR-deleted ISAV may only be granted to a Member State, a zone or a compartment with an unknown health status with regard to infection with HPR-deleted ISAV if all establishments and, when required, selected sampling points in wild populations selected in accordance with (b) of Section 1, have been subject to the following scheme:

(a) the establishments or sampling points have been subject to health visits and sampled for a minimum period of 2 consecutive years as laid down in Table 2.A;

(b) during that 2-year period, the testing of all samples using the diagnostic methods set out in point 2 of Section 5 must have produced negative results for HPR-deleted ISAV and any suspicion of infection must have been ruled out in accordance with the diagnostic methods set out in point 3 of Section 5;

(c) If infection with HPR-deleted ISAV is detected during the surveillance referred to in point (a); before re-starting the scheme, relevant establishments within the Member State, zone or compartment must:

(i) be subject to the minimum disease control measures laid down in Articles 58 to 65;

(ii) be repopulated with fish from an establishment in a Member State, zone or compartment free from infection with HPR-deleted ISAV or from an establishment in a Member State, zone or compartment covered by an eradication programme for that disease.

Table 2.A

Scheme for Member States, zones and compartments for the 2-year control period which precedes the achievement of status free from infection with HPR-deleted ISAV

Year of surveillance	Number of health visits per year to each establishment	Number of laboratory examinations per year(1)	Number of fish in the sample
Year 1	6	2	75
Year 2	6	2	75

(1) Samples must be collected during spring and autumn each year.

(2) Maximum number of fish per pool: 5.

SECTION 3
GRANTING OF THE STATUS FREE FROM INFECTION WITH HPR-DELETED ISAV IN MEMBER STATES, ZONES AND COMPARTMENTS KNOWN TO BE INFECTED WITH HPR-DELETED ISAV

1. The status free from infection with HPR-deleted ISAV may only be granted to a Member State, a zone or a compartment known to be infected with HPR-deleted ISAV if all establishments keeping listed species within the Member State, zone or compartment have been subject to an eradication programme that complies with the following requirements:

   (a) the minimum control measures laid down in Articles 55 to Article 65 have been applied and a restricted zone of an appropriate size as provided for in point (c) of Article 58(1), where appropriate, divided into a protection zone and a surveillance zone, must have been established in the vicinity of the establishment(s) infected with HPR-deleted ISAV, taking into account the requirements set out in point 2;

   (b) all establishments keeping listed species within the protection zone, or where a protection zone has not been established, the restricted zone, not infected with HPR-deleted ISAV must be subject to an investigation comprising at least the following elements:

      (i) the collection of samples for testing of minimum 10 moribund fish, when clinical signs or *post-mortem* lesions consistent with infection with HPR-deleted ISAV are observed, or minimum 30 fish when clinical signs or *post mortem* lesions are not observed;

      (ii) in those establishments where the tests referred to in (i) have produced negative results, the health visits must continue once per month until the protection zone is withdrawn in accordance with point (c);

(c) relevant establishments must be emptied in accordance with Article 62, cleaned and disinfected in accordance with Article 63 and fallowed in accordance with Article 64.

The duration of the fallowing period referred to in point (b) of Article 64(2) shall be at least 3 months. When all establishments infected within the same protection zone, or where a protection zone has not been established, the restricted zone, are emptied, at least 6 weeks of synchronised fallowing must be carried out.

When fallowing of the infected establishments is carried out, the restricted zone or the protection zone, when it has been established, must be converted into a surveillance zone until the scheme set out in Section 2 is completed;

(d) repopulation may only take place when all infected establishments have been emptied, cleaned, disinfected and fallowed in accordance with point (c);

(e) all establishments other than those referred to in point (f) which keep listed species within the Member State, zone or compartment covered by the eradication programme and when surveillance in wild populations is required, all sampling points selected in accordance with point (b) of Section 1, must subsequently be subject to the scheme set out in Section 2;

(f) an individual establishment which keeps listed species and which has a health status which is independent of the health status of the surrounding waters is not required to comply with the scheme set out in Section 2 following a disease outbreak provided the establishment complies with the requirements set out in paragraph 3 of Article 80 and is re-populated with fish sourced from Member States, zones or compartments with status free from infection with HPR-deleted ISAV.

2. The restricted zone must have been defined on a case-by-case basis and:

(a) it must take into account factors influencing the risks for the spread of infection with HPR-deleted ISAV to kept and wild fish, such as:

(i) the number, rate and distribution of the mortalities on the establishment infected with HPR-deleted ISAV or in other aquaculture establishments;

(ii) the distance to and density of neighbouring establishments;

(iii) the proximity to slaughterhouses;

(iv) contact establishments;

(v) species present at the establishments;

(vi) the farming practices applied in the infected establishments and in the neighbouring establishments to the infected establishment;

(vii) the hydrodynamic conditions; and

(viii) other factors of epidemiological significance identified;

(b) the geographical demarcation in coastal areas must comply with the following minimum requirements:

(i) the protection zone must consist of an area included in a circle with a radius of at least one tidal excursion or at least 5 km, whichever is larger, centred on the establishment infected with HPR-deleted ISAV, or an

equivalent area determined according to appropriate hydrodynamic or epidemiological data; and

(ii) the surveillance zone must consist of an area surrounding the protection zone, of overlapping tidal excursion zones; or an area surrounding the protection zone and included in a circle of radius 10km from the centre of the protection zone; or an equivalent area determined according to appropriate hydrodynamic or epidemiological data;

or

(iii) where separate protection and surveillance zones are not established, the restricted zone must consist of an area comprising both the protection zone and the surveillance zone;

(c) the geographical demarcation in inland areas must comprise the entire water catchment area in which the establishment infected with HPR-deleted ISAV is located. The competent authority may limit the extent of the restricted zone to parts of the water catchment area, provided this limitation does not compromise the disease control measures with respect to infection with HPR-deleted ISAV.

SECTION 4
MAINTENANCE OF STATUS FREE FROM INFECTION WITH HPR-DELETED ISAV

1. When targeted surveillance is required in order to maintain the status free from infection with HPR-deleted ISAV of a Member State, a zone or a compartment, in accordance with Article 81, all establishments keeping listed species within the Member State, zone or compartment concerned must be subject to health visits and fish must be sampled in accordance with Table 2.B, taking into account the risk level of the establishment for the contraction of infection with HPR-deleted ISAV.

2. When determining the frequency of health visits required to maintain the status free from infection with HPR-deleted ISAV of compartments where the health status is dependent on the health status of the aquatic animal population in surrounding natural waters, the risk for the contraction of infection with HPR-deleted ISAV must be regarded as high.

3. Disease-free status must only be maintained as long as all samples tested, using the diagnostic methods set out in point 2 of Section 5, have produced negative results for HPR-deleted ISAV and any suspicion of infection with HPR-deleted ISAV has been ruled out in accordance with the diagnostic methods set out in point 3 of Section 5.

Table 2.B

Scheme for Member States, zones or compartments to maintain status free from infection with HPR-deleted ISAV(1)

Risk level(2)	**Number of health visits per year**	**Number of laboratory examinations per year**(3,4)	**Number of fish in the sample**
High	2	2	30
Medium	1	1	30

Low	1 every 2 years	1 every 2 years	30

(1) Shall not apply to establishments rearing only rainbow trout (*Oncorhynchus mykiss*) or brown trout (*Salmo trutta*) or both rainbow trout and brown trout, and where the water supply is exclusively based on fresh water sources which are not populated with Atlantic salmon (*Salmo salar*).

(2) Risk level assigned to the establishment by the competent authority as set out in paragraph 1 of Chapter 2 of Part I other than in the case of dependent compartments where all establishments are deemed to be high risk.

(3) Samples must be collected during spring and autumn when two samples are required each year

(4) Samples must be collected during spring or autumn when one sample per year is required.

(5) Maximum number of fish per pool: 5

SECTION 5
DIAGNOSTIC AND SAMPLING METHODS

1. The organs or tissue material to be sampled and examined must be:

   (a) Histology: anterior-kidney, liver, heart, pancreas, intestine, spleen and gill;

   (b) Immunohistochemistry: mid-kidney and heart including valves and *bulbus arteriosus*;

   (c) RT-qPCR analysis: mid-kidney and heart;

   (d) Virus culture: mid-kidney, heart, liver and spleen.

   Organ pieces from a maximum of five fish may be pooled.

2. The diagnostic method to be used to grant or to maintain status free from infection with HPR-deleted ISAV in accordance with Sections 2 to 4 must be RT-qPCR, followed by conventional RT-PCR and sequencing of the HE-gene of positive samples in accordance with the detailed methods and procedures which must be those approved by the EURL for fish diseases.

   In the case of a positive RT-qPCR result, further samples must be tested before the implementation of the initial control measures provided for in Articles 55 to 65.

   Those samples must be tested as follows in accordance with the detailed methods and procedures approved by the EURL for fish diseases:

   (a) screening of the samples by RT-qPCR, followed by conventional RT-PCR and sequencing of the HE-gene to verify HPR-deletion; and

   (b) detection of ISAV antigen in tissue preparations by means of specific antibodies against ISAV; or

   (c) isolation in cell culture and subsequent identification of HPR-deleted ISAV.

3. When a suspicion of infection with HPR-deleted ISAV must be confirmed or ruled out in accordance with Article 55, the following visit, sampling and testing procedure must comply with the following requirements:

   (a) the suspected establishment must be subject to at least one health visit and one sampling of 10 moribund fish, when clinical signs or *post-mortem* lesions consistent with infection with HPR-deleted ISAV are observed, or minimum 30 fish when clinical signs or *post-mortem* lesions are not observed. Samples shall be tested using one or more of the diagnostic methods set out in point 2 in accordance with the detailed diagnostic methods and procedures approved by the EURL for fish diseases;

(b) in the case of a positive result of RT-qPCR for HPR-deleted ISAV, further samples shall be tested before the implementation of the initial control measures provided in Article 58. A suspected case of infection with HPR-deleted ISAV shall be confirmed in accordance with the following criteria using the detailed methods and procedures approved by the EURL for fish diseases:

(i) Detection of ISAV by RT-qPCR, followed by sequencing of the HE-gene to verify HPR-deletion, and detection of ISAV in tissue preparations by means of specific antibodies against ISAV;

(ii) detection of ISAV by RT-qPCR, including sequencing of the HE-gene to verify HPR-deletion; and isolation and identification of ISAV in cell culture from at least one sample from any fish from the establishment;

(c) where the presence of clinical, gross pathological or histopathological findings consistent with infection are observed, the findings must be corroborated by virus detection by two diagnostic methods with independent principles of detection, such as RT-qPCR and IHC, in accordance with the procedures approved by the EURL for fish diseases.

The suspicion of HPR-deleted ISAV may be ruled out, if tests and health visits over a period of 12 months from the date of the suspicion are found to reveal no further evidence of the presence of the virus.

Chapter 3
Eradication, disease-free status and diagnostic methods for infection with *Marteilia refringens*

SECTION 1
GENERAL REQUIREMENTS FOR HEALTH VISITS AND SAMPLING

Health visits and sampling for the surveillance referred to in point (b)(ii) of Article 3(2) must comply with the following requirements:

(a) health visits and, where appropriate, the sampling must be carried out in the period of the year when prevalence of the parasite in the Member State, zone or compartment is known to be maximal. When such data is not available, sampling must be carried out just after the water temperature has exceeded 17°C;

(b) when molluscs must be sampled in accordance with the requirements set out in Sections 2 to 4, the following selection criteria must apply:

(i) if *Ostrea* spp. are present, only oysters of that species must be selected for sampling. If *Ostrea* spp. are not present, the sample must be representative of all other susceptible species present;

(ii) if weak, gaping or freshly dead but not decomposed molluscs are present in the production units, such molluscs must primarily be selected. If such molluscs are not present, the molluscs selected must include the oldest healthy molluscs;

(iii) when sampling in mollusc establishments which utilise more than one water source for mollusc production, molluscs representing all water sources must be

included for sampling in such a way that all parts of the establishment are proportionally represented in the sample;

(iv) when sampling in mollusc establishments or groups of establishments, molluscs from a sufficient number of sampling points, must be included in the sample in such a way that all parts of the establishment or group of establishments are proportionally represented in the sample. The main factors to be considered for the selection of these sampling points are previous sampling points where *Marteilia refringens* was detected, stocking density, water flows, presence of susceptible species, presence of vector species, bathymetry and management practices. Natural beds within or adjacent to the establishment or group of establishments must be included in the sampling.

SECTION 2

GRANTING OF THE STATUS FREE FROM INFECTION WITH *MARTEILIA REFRINGENS* IN MEMBER STATES, ZONES AND COMPARTMENTS OF UNKNOWN HEALTH STATUS

1. The status free from infection with *Marteilia refringens* may only be granted to a Member State, a zone or a compartment with an unknown health status with regard to infection with *Marteilia refringens* if all establishments or groups of establishments keeping listed species within the Member State, zone or compartment and where required, sampling points in wild populations, have been subject to the following 3-year scheme:

   (a) the establishments or groups of establishments keeping listed species have been subject to health visits and sampled for a minimum period of 3 consecutive years as laid down in Table 3.A;

   (b) during that 3-year period, the testing of all samples using the diagnostic methods set out in point 2 of Section 5 have produced negative results for *Marteilia refringens* and any suspicion of *Marteilia refringens* has been ruled out in accordance with the diagnostic methods set out in point 3 of Section 5;

   (c) when *Ostrea edulis* sourced from a Member State, zone or compartment of disease-free status are to be included in the sample, they must have been introduced into the establishment or group of establishments at least in the spring just preceding the period when the scheme is carried out.

2. If *Marteilia refringens* is detected during the 3-year scheme set out in point 1, before starting a new 3-year scheme, relevant establishments in the Member State, zone or compartment must:

   (a) be subject to the minimum disease control measures laid down in Articles 58 to 65;

   (b) be repopulated with molluscs from an establishment in a Member State, zone or compartment free from infection with *Marteilia refringens* or from an establishment in a Member State, zone or compartment covered by an eradication programme for that disease.

Table 3.A

Scheme for Member States, zones or compartments for the 3-year control period which precedes the achievement of status free from infection with *Marteilia refringens*

Year of surveillance	Number of health visits per year to each establishment/ group of establishments	Number of laboratory examinations per year	Number of molluscs in the sample
Year 1	1	1	150
Year 2	1	1	150
Year 3	1	1	150

SECTION 3
GRANTING OF THE STATUS FREE FROM INFECTION WITH *MARTEILIA REFRINGENS* IN MEMBER STATES, ZONES AND COMPARTMENTS KNOWN TO BE INFECTED WITH *MARTEILIA REFRINGENS*

1. The status free from infection with *Marteilia refringens* may only be granted to a Member State, a zone or a compartment known to be infected with *Marteilia refringens,* where the competent authority judges that eradication of this disease to be feasible, if all establishments or groups of establishments keeping listed species within that Member State, zone or compartment have been subject to an eradication programme that complies with the following requirements:

   (a) the minimum control measures laid down in Articles 55 to 65 have effectively been applied and a restricted zone of an appropriate size as provided for in point (c) of Article 58(1), where appropriate divided into a protection zone and surveillance zone, must have been established in the vicinity of the establishment(s) or group of establishments infected with *Marteilia refringens*, taking into account the requirements set out in point 2;

   (b) all establishments and groups of establishments keeping listed species within the protection zone, or where a protection zone has not been established, the restricted zone, not infected with *Marteilia refringens* must be subject to an investigation comprising at least the collection of samples for the testing of 150 molluscs after the beginning of the transmission period of *Marteilia refringens*. When the transmission period is not known, the sampling must begin in the period after the temperature of the water exceeds 17 °C;

   (c) relevant establishments and groups of establishments must be emptied in accordance with Articles 62, and if possible cleaned and disinfected in accordance with Article 63.

   Fallowing must be carried out in accordance with Article 64 and the duration of the fallowing period must be at least:

   (i) 2 months in case of the establishments and groups of establishments which can be fully drained and thoroughly cleaned and disinfected such as hatcheries and nurseries;

(ii) 2 months in case of the establishments and groups of establishments which cannot be drained and thoroughly cleaned and disinfected provided that the infected molluscs of the listed species and those molluscs of the listed species with epidemiological links with the infected establishment or group of establishments have been harvested or removed before the period of the year when the prevalence of *Marteilia refringens* is known to be maximal, or when that period is not known, before the period when water temperature exceeds 17 °C;

(iii) 14 months in case of the establishments and groups of establishments which cannot be drained and thoroughly cleaned and disinfected if the infected molluscs of the listed species and those molluscs of the listed species with epidemiological links with the infected establishment or group of mollusc establishments have not been harvested or removed before the period of the year when the prevalence of *Marteilia refringens* is known to be maximal or when such data is not known, when molluscs of the susceptible species have not been harvested or removed before the period when water temperature exceeds 17 °C.

When all infected establishments and infected groups of establishments are emptied, at least 4 weeks of synchronised fallowing must be carried out;

(d) repopulation may only take place when all infected establishments or infected groups of establishments have been emptied, cleaned, disinfected and fallowed in accordance with point (c);

(e) all establishments and groups of establishments other than those referred to in point (f) which keep listed species within the Member State, zone or compartment covered by the eradication programme, must subsequently be subject to the scheme set out in Section 2;

(f) an individual establishment which keeps listed species and which has a health status which is independent of the health status of the surrounding waters is not required to comply with the scheme set out in Section 2 following a disease outbreak provided the establishment complies with the requirements set out in paragraph 3 of Article 80 and is repopulated with molluscs sourced from Member States, zones or compartments with status free from infection with *Marteilia refringens*.

2. The restricted zone must have been defined on a case-by-case basis and:

(a) it must take into account factors influencing the risks for the spread of infection with *Marteilia refringens* including other establishments and wild molluscs, such as:

(i) the number, age, rate and distribution of the mortalities of molluscs on the establishment or group of establishments infected with *Marteilia refringens;*

(ii) the distance to and density of neighbouring establishments or groups of establishments and wild molluscs;

(iii) the proximity to processing establishments, contact establishments or groups of establishments;

(iv) the species, especially susceptible species and vector species, present at the establishments or groups of establishments;

(v) the farming practices applied in the affected and neighbouring establishments and groups of establishments;

(vi) the hydrodynamic conditions; and

(vii) other factors of epidemiological significance identified;

(b) the geographical demarcation must comply with the following minimum requirements:

(i) the protection zone must consist of an area included in a circle with a radius of at least one tidal excursion or at least 5 km, whichever is larger, centred on the establishment infected with *Marteilia refringens*, or an equivalent area determined according to appropriate hydrodynamic or epidemiological data; and

(ii) the surveillance zone must consist of an area surrounding the protection zone, of overlapping tidal excursion zones; or an area surrounding the protection zone and included in a circle of radius 10km from the centre of the protection zone; or an equivalent area determined according to appropriate hydrodynamic or epidemiological data;

or

(iii) where separate protection and surveillance zones are not established, the restricted zone must consist of an area comprising both the protection zone and the surveillance zone.

SECTION 4

MAINTENANCE OF STATUS FREE FROM INFECTION WITH *MARTEILIA REFRINGENS*

1. When targeted surveillance is required in order to maintain the status free from infection with *Marteilia refringens* of a Member State, a zone or a compartment, in accordance with Article 81, all establishments keeping listed species within the Member State, zone or compartment concerned must be subject to health visits and molluscs must be sampled in accordance with Table 3.B, taking into account the risk level of the establishment for the contraction of infection with *Marteilia refringens*.

2. When determining the frequency of health visits required to maintain the status free from infection with *Marteilia refringens* of compartments, where the health-status regarding that disease is dependent on the health-status of the aquatic animal populations in surrounding natural waters, the risk for the contraction of infection with *Marteilia refringens* must be regarded as high.

3. The status free from infection with *Marteilia refringens* may only be maintained as long as all samples tested, using the diagnostic methods set out in point 2 of Section 5 have produced negative results for *Marteilia refringens* and any suspicion of infection with *Marteilia refringens* has been ruled out in accordance with the diagnostic methods set out in point 3 of section 5.

Table 3.B

Scheme for Member States, zones or compartments to maintain disease-free status for *Marteilia refringens*

Risk level(1)	Number of health visits to each establishment/ group of establishments	Number of laboratory examinations	Number of molluscs in the sample
High	1 every year	1 every 2 years	150
Medium	1 every 2 years	1 every 2 years	150
Low	1 every 3 years	1 every 3 years	150

(1) Risk level assigned to the establishment by the competent authority as set out in paragraph 1 of Chapter 2 of Part I other than in the case of dependent compartments where all establishments are deemed to be high risk.

SECTION 5
DIAGNOSTIC AND SAMPLING METHODS

1. The whole animal must be submitted to the laboratory for the performance of the diagnostic tests provided for in points 2 and 3.

2. The diagnostic methods to be used to grant or maintain status free from infection with *Marteilia refringens* in accordance with Sections 2 to 4 must follow the detailed diagnostic methods and procedures approved by the EURL for Mollusc Diseases and must be histopathology, tissue imprints or PCR.

3. When a suspicion of infection with *Marteilia refringens* is required to be confirmed or ruled out in accordance with Article 55 the following visit, sampling and testing procedure must be complied with:

   (a) the investigation must include at least one sampling of 30 molluscs of susceptible species if the suspicion is based on a mortality report or if not, 150 molluscs of susceptible species after the beginning of the transmission period of *Marteilia refringens*. When the transmission period is not known, the sampling must begin in the period after the temperature of the water exceeds 17 °C;

   (b) samples must be tested using the diagnostic methods set out in point (i) following the detailed diagnostic methods and procedures approved by the EURL for Mollusc Diseases:

      (i) the presence of *Marteilia refringens* must be considered as confirmed when a positive result by histopathology, tissue imprints or *in situ* hybridisation is combined with a positive PCR result completed by sequencing. If biological material is not available for histopathology, tissue imprints or *in situ* hybridisation, the presence of *Marteilia refringens* must be considered as confirmed when positive results are obtained using two PCR assays targeting different fragments of the parasite genome and completed by sequencing;

(ii) the suspicion of infection with *Marteilia refringens* may be ruled out, if the tests referred to in (i) reveal no further evidence of the presence of *Marteilia refringens*.

Chapter 4
Eradication, disease-free status and diagnostic methods for infection with *Bonamia exitiosa*

SECTION 1
GENERAL REQUIREMENTS FOR HEALTH VISITS AND SAMPLING

Health visits and sampling for the surveillance referred to in point (b)(ii) of Article 3(2) must comply with the following requirements:

(a) health visits and, where appropriate, the sampling must be carried out in the period of the year when prevalence of the parasite in the Member State, zone or compartment is known to be maximal. When such data is not available, sampling shall be carried out twice a year, in spring and autumn;

(b) when molluscs are to be sampled in accordance with the requirements set out in Sections 2 to 4, the following criteria must apply:

(i) if *Ostrea* spp. are present, only oysters of that species must be selected for sampling. If *Ostrea* spp. are not present, the sample must be representative of all other susceptible species present;

(ii) if weak, gaping or freshly dead but not decomposed molluscs are present, such molluscs must primarily be selected. If such molluscs are not present, the molluscs selected must include the oldest healthy molluscs;

(iii) when sampling in establishments or groups of establishments which utilise more than one water source for mollusc production, molluscs representing all water sources must be included for sampling in such a way that all parts of the establishment are proportionally represented in the sample;

(iv) when sampling in mollusc establishments or groups of establishments, molluscs from a sufficient number of sampling points must be included in the sample in such a way that all parts of the establishment or group of establishments are proportionally represented in the sample. The main factors to be considered for the selection of those sampling points are previous points where *Bonamia exitiosa* was detected, stocking density, water flows, the presence of susceptible species, the presence of vector species (e.g.*Crassostrea gigas*), bathymetry and management practices. Natural beds within or adjacent to the establishment or group of establishments shall be included in the sampling.

SECTION 2
GRANTING OF THE STATUS FREE FROM INFECTION WITH *BONAMIA EXITIOSA* IN MEMBER STATES, ZONES AND COMPARTMENTS OF UNKNOWN HEALTH STATUS

1. The status free from infection with *Bonamia exitiosa* may only be granted to a Member State, a zone or a compartment with an unknown health status with regard

to infection with *Bonamia exitiosa* if all establishments or groups of establishments keeping listed species within the Member State, zone or compartment and where required, sampling points in wild populations, have been subject to the following 3-year scheme:

(a) the establishments and groups of establishments keeping listed species have been subject to health visits and sampled for a minimum period of 3 consecutive years as laid down in Table 4.A;

(b) during that 3-year period, the testing of all samples using the diagnostic methods set out in point 2 of Section 5 have produced negative results for *Bonamia exitiosa* and any suspicion of *Bonamia exitiosa* has been ruled out in accordance with the diagnostic methods set out in point 3 of Section 5;

(c) when *Ostrea edulis* sourced from a Member State, zone or compartment of disease-free status are to be included in the sample, they must have been introduced into the establishment or group of establishments at least one year before the scheme is carried out.

2. If infection with *Bonamia exitiosa* is detected during the 3-year scheme referred to in point 1; before starting a new 3-year scheme, relevant establishments in the Member State, zone or compartment must:

(a) be subject to the minimum disease control measures laid down in Articles 58 to 65;

(b) be repopulated with molluscs from an establishment in a Member State, zone or compartment free from infection with *Bonamia exitiosa* or from an establishment in a Member State, zone or compartment covered by an eradication programme for that disease.

Table 4.A

Scheme for Member States, zones or compartments for the 3-year control period which precedes the achievement of status free from infection with *Bonamia exitiosa*

Year of surveillance	Number of health visits per year to each establishment or group of establishments	Number of laboratory examinations per year	Number of molluscs in the sample
Year 1	2	2	150
Year 2	2	2	150
Year 3	2	2	150

SECTION 3

GRANTING OF THE STATUS FREE FROM INFECTION WITH *BONAMIA EXITIOSA* IN MEMBER STATES, ZONES AND COMPARTMENTS KNOWN TO BE INFECTED WITH *BONAMIA EXITIOSA*

1. The status free from infection with *Bonamia exitiosa* may only be granted to a Member State, a zone or a compartment known to be infected with *Bonamia exitiosa,* where the competent authority judges that eradication of this disease to be feasible, if all establishments or groups of establishments keeping listed species within that

Member State, zone or compartment have been subject to an eradication programme that complies with the following requirements:

(a) the minimum control measures laid down in Articles 55 to 65 must have been effectively applied, and a restricted zone of an appropriate size as provided for in point (c) of Article 58(1), where appropriate, divided into a protection zone and surveillance zone; must have been established in the vicinity of the establishment or group of establishments declared infected with *Bonamia exitiosa* taking into account the requirements set out in point 2;

(b) all establishments and groups of establishments keeping listed species within the protection zone or where a protection zone has not been established, within the restricted zone, not infected with *Bonamia exitiosa* must be subject to an investigation comprising at least the collection of samples for testing of 150 molluscs of susceptible species after the beginning of the transmission period of *Bonamia exitiosa*. When the transmission period is not known, the sampling must be done on oysters which have spent at least one year within the protection zone;

(c) relevant establishments and groups of establishments must be emptied in accordance with Article 62, and if possible, cleaned and disinfected in accordance with Article 63.

Fallowing must be carried out in compliance with Article 64 and the duration of the fallowing period must be at least 6 months.

When all infected establishments or infected groups of establishments are emptied, at least 4 weeks of synchronised fallowing must be carried out;

(d) repopulation may only take place when all infected establishments or infected groups of establishments have been emptied, cleaned, disinfected and fallowed in accordance with point (c);

(e) all establishments and groups of establishments other than those referred to in point (f) which keep listed species within the Member State, zone or compartment covered by the eradication programme, must subsequently be subject to the scheme set out in Section 2;

(f) an individual establishment which keeps listed species and which has a health-status which is independent of the health-status of the surrounding waters is not required to comply with the scheme set out in Section 2 following a disease outbreak provided the establishment complies with the requirements set out in paragraph 3 of Article 80 and is repopulated with molluscs sourced from Member States, zones or compartments with status free from infection with *Bonamia exitiosa*.

2. The restricted zone must have been defined on a case-by-case basis and:

(a) it must take into account factors influencing the risks for the spread of infection with *Bonamia exitiosa* including other establishments and wild molluscs, such as:

(i) the number, age, rate and distribution of the mortalities of molluscs on the establishment or group of establishments infected with *Bonamia exitiosa*;

(ii) the distance to and density of neighbouring establishments or groups of establishments and wild molluscs;

(iii) the proximity to processing establishments, contact establishments or groups of establishments;

(iv) the species, especially susceptible species and vector species, present at the establishments or groups of establishments;

(v) the farming practices applied in the affected and neighbouring establishments and groups of establishments;

(vi) the hydrodynamic conditions; and

(vii) other factors of epidemiological significance identified;

(b) the geographical demarcation must comply with the following minimum requirements:

(i) the protection zone must consist of an area included in a circle with a radius of at least one tidal excursion or at least 5 km, whichever is larger, centred on the establishment infected with *Bonamia exitiosa*, or an equivalent area determined according to appropriate hydrodynamic or epidemiological data; and

(ii) the surveillance zone must consist of an area surrounding the protection zone, of overlapping tidal excursion zones; or an area surrounding the protection zone and included in a circle of radius 10km from the centre of the protection zone; or an equivalent area determined according to appropriate hydrodynamic or epidemiological data;

or

(iii) where separate protection and surveillance zones are not established, the restricted zone must consist of an area comprising both the protection zone and the surveillance zone.

SECTION 4

MAINTENANCE OF STATUS FREE FROM INFECTION WITH *BONAMIA EXITIOSA*

1. When targeted surveillance is required in order to maintain the status free from infection with *Bonamia exitiosa* of a Member State, a zone or a compartment, in accordance with Article 81, all establishments keeping listed species within the Member State, zone or compartment concerned must be subject to health visits and molluscs must be sampled in accordance with Table 4.B, taking into account the risk level of the establishment for the contraction of infection with *Bonamia exitiosa*

2. When determining the frequency of health visits required to maintain the status free from infection with *Bonamia exitiosa* of compartments where the health status regarding that disease is dependent on the health status of the aquatic animal populations in surrounding natural waters, the risk for the contraction of infection with *Bonamia exitiosa* must be regarded as high.

3. The status free from infection with *Bonamia exitiosa* may only be maintained as long as all samples, using the diagnostic methods set out in point 2 of Section 5 have produced negative results for *Bonamia exitiosa* and any suspicion of infection with

Bonamia exitiosa has been ruled out in accordance with the diagnostic methods set out in point 3 of Section 5.

Table 4.B

Scheme for Member States, zones or compartments to maintain status free from infection with *Bonamia exitiosa*

Risk level(1)	Number of health visits to each establishment/ group of establishments	Number of laboratory examinations	Number of molluscs in the sample
High	1 every year	1 every 2 years	150
Medium	1 every 2 years	1 every 2 years	150
Low	1 every 3 years	1 every 3 years	150

(1) Risk level assigned to the establishment by the competent authority as set out in paragraph 1 of Chapter 2 of Part I other than in the case of dependent compartments where all establishments are deemed to be high risk.

SECTION 5
DIAGNOSTIC AND SAMPLING METHODS

1. The whole animal must be submitted to the laboratory for the performance of the diagnostic tests provided for in points 2 and 3.

2. The diagnostic methods to be used to grant or maintain status free from infection with *Bonamia exitiosa,* in accordance with Sections 2 to 4 must follow the detailed diagnostic methods and procedures approved by the EURL for Mollusc Diseases and must be histopathology, tissue imprints or PCR.

3. When a suspicion of infection with *Bonamia exitiosa* is required to be confirmed or ruled out in accordance with Article 58, the following visit, sampling and testing procedure must be complied with:

   (a) the investigation must include at least one sampling of 30 molluscs of susceptible species if the suspicion is based on a mortality report, or if not, 150 molluscs of susceptible species after the beginning of the transmission period of *Bonamia exitiosa*. When the transmission period is not known, the sampling shall be carried out twice a year, in spring and autumn;

   (b) the samples must be tested using the diagnostic methods set out in point (i) following the detailed diagnostic methods and procedures which have been approved by the EURL for Mollusc Diseases:

      (i) the presence of *Bonamia exitiosa* must be considered as confirmed when a positive result by histopathology, tissue imprints or *in situ* hybridisation is combined with a positive result by PCR followed by sequencing. If biological material is not available for histopathology, tissue imprints or *in situ* hybridisation, the presence of *Bonamia exitiosa* must be considered as confirmed when positive results are obtained using two PCR assays targeting different fragments of the parasite genome and completed by sequencing;

      (ii) the suspicion of the presence of infection with *Bonamia exitiosa* must be ruled out, if those tests reveal no further evidence of the presence of *Bonamia exitiosa*.

Chapter 5
Eradication, disease-free status and diagnostic methods for infection with *Bonamia ostreae*

SECTION 1
GENERAL REQUIREMENTS FOR HEALTH VISITS AND SAMPLING

Health visits and sampling for the surveillance referred to in point (b)(ii) of Article 3(2) must comply with the following requirements:

(a) health visits and, where appropriate, the sampling must be carried out in the period of the year when prevalence of the parasite in the Member State, zone or compartment is known to be maximal. When such data is not available, sampling must be carried out in winter or at the beginning of spring;

(b) when molluscs are to be sampled in accordance with the requirements set out in Sections 2 to 4, the following criteria must apply:

  (i) if *Ostrea edulis* are present, only oysters of that species must be selected for sampling. If *Ostrea edulis* are not present, the sample must be representative of all other susceptible species present;

  (ii) if weak, gaping or freshly dead but not decomposed molluscs are present, such molluscs must primarily be selected. If such molluscs are not present, the molluscs selected must include the oldest healthy molluscs;

  (iii) when sampling in establishments or groups of establishments which utilise more than one water source for mollusc production, molluscs representing all water sources must be included for sampling in such a way that all parts of the establishment are proportionally represented in the sample;

  (iv) when sampling in mollusc establishments or groups of establishments, molluscs from a sufficient number of sampling points must be included in the sample in such a way that all parts of the establishment or group of establishments are proportionally represented in the sample. The main factors to be considered for the selection of those sampling points are previous points where *Bonamia ostreae* was detected, stocking density, water flows, the presence of susceptible species, the presence of vector species, bathymetry and management practices. Natural beds within or adjacent to the establishment or group of establishments shall be included in the sampling.

SECTION 2
GRANTING OF THE STATUS FREE FROM INFECTION WITH *BONAMIA OSTREAE* IN MEMBER STATES, ZONES AND COMPARTMENTS OF UNKNOWN HEALTH STATUS

1. The status free from infection with *Bonamia ostreae* may only be granted to a Member State, a zone or a compartment with an unknown health status with regard to infection with *Bonamia ostreae* if all establishments or groups of establishments keeping listed species within the Member State, zone or compartment and where required, sampling points in wild populations, have been subject to the following 3-year scheme:

(a) the establishments and groups of establishments keeping listed species have been subject to health visits and sampled for a minimum period of 3 consecutive years as laid down in Table 5.A;

(b) during that 3-year period, the testing of all samples using the diagnostic methods set out in point 2 of Section 5 have produced negative results for *Bonamia ostreae* and any suspicion of *Bonamia ostreae* has been ruled out in accordance with the diagnostic methods set out in point 3 of Section 5;

(c) when *Ostrea edulis* sourced from a Member State, zone or compartment of disease-free status are to be included in the sample, they must have been introduced into the establishment or group of establishments at least one year before the scheme is carried out.

2. If infection with *Bonamia ostreae* is detected during the 3-year scheme referred to in point 1; before starting a new 3-year scheme, relevant establishments in the Member State, zone or compartment must:

(a) be subject to the minimum disease control measures laid down in Articles 58 to 65;

(b) be repopulated with molluscs from an establishment in a Member State, zone or compartment free from infection with *Bonamia ostreae* or from an establishment in a Member State, zone or compartment covered by an eradication programme for that disease.

SECTION 3

GRANTING OF THE STATUS FREE FROM INFECTION WITH *BONAMIA OSTREAE* IN MEMBER STATES, ZONES AND COMPARTMENTS KNOWN TO BE INFECTED WITH *BONAMIA OSTREAE*

1. The status free from infection with *Bonamia ostreae* may only be granted to a Member State, a zone or a compartment known to be infected with *Bonamia ostreae,* where the competent authority judges that eradication of this disease to be feasible, if all establishments or groups of establishments keeping listed species within that Member State, zone or compartment have been subject to an eradication programme that complies with the following requirements:

(a) the minimum control measures laid down in Articles 55 to 65 must have been effectively applied, and a restricted zone of an appropriate size as provided for in point (c) of Article 58(1), where appropriate, divided into a protection zone and surveillance zone; must have been established in the vicinity of the establishment or group of establishments declared infected with *Bonamia ostreae* taking into account the requirements set out in point 2;

(b) all establishments and groups of establishments keeping listed species within the protection zone or where a protection zone has not been established, within the restricted zone, not infected with *Bonamia ostreae* must be subject to an investigation comprising at least the collection of samples for testing of 150 molluscs of susceptible species after the beginning of the transmission period of *Bonamia ostreae* . When the transmission period is not known, the sampling must begin in winter or at the beginning of spring;

(c) relevant establishments and groups of establishments must be emptied in accordance with Article 62, and if possible, cleaned and disinfected in accordance with Article 63.

Fallowing must be carried out in compliance with Article 64 and the duration of the fallowing period must be at least 6 months.

When all infected establishments or infected groups of establishments are emptied, at least 4 weeks of synchronised fallowing must be carried out;

(d) repopulation may only take place when all infected establishments or infected groups of establishments have been emptied, cleaned, disinfected and fallowed in accordance with point (c);

(e) all establishments and groups of establishments other than those referred to in point (f) which keep listed species within the Member State, zone or compartment covered by the eradication programme, must subsequently be subject to the scheme set out in Section 2;

(f) an individual establishment which keeps listed species and which has a health status which is independent of the health status of the surrounding waters is not required to comply with the surveillance scheme set out in Section 2 following a disease outbreak provided the establishment complies with the requirements set out in paragraph 3 of Article 80 and is repopulated with molluscs sourced from Member States, zones or compartments with status free from infection with *Bonamia ostreae*.

2. The restricted zone must have been defined on a case-by-case basis and:

(a) it must take into account factors influencing the risks for the spread of infection with *Bonamia ostreae* including other establishments and wild molluscs, such as:

(i) the number, age, rate and distribution of the mortalities of molluscs on the establishment or group of establishments infected with *Bonamia ostreae*;

(ii) the distance to and density of neighbouring establishments or groups of establishments and wild molluscs;

(iii) the proximity to processing establishments, contact establishments or groups of establishments;

(iv) the species, especially susceptible species and vector species, present at the establishments or groups of establishments;

(v) the farming practices applied in the affected and neighbouring establishments and groups of establishments;

(vi) the hydrodynamic conditions; and

(vii) other factors of epidemiological significance identified;

(b) the geographical demarcation must comply with the following minimum requirements:

(i) the protection zone must consist of an area included in a circle with a radius of at least one tidal excursion or at least 5 km, whichever is larger, centred on the establishment infected with *Bonamia ostreae*, or an

equivalent area determined according to appropriate hydrodynamic or epidemiological data; and

(ii) the surveillance zone must consist of an area surrounding the protection zone, of overlapping tidal excursion zones; or an area surrounding the protection zone and included in a circle of radius 10km from the centre of the protection zone; or an equivalent area determined according to appropriate hydrodynamic or epidemiological data;

or

(iii) where separate protection and surveillance zones are not established, the restricted zone must consist of an area comprising both the protection zone and the surveillance zone.

Table 5.A

Scheme for Member States, zones or compartments for the 3-year control period which precedes the achievement of status free from infection with *Bonamia ostreae*

Year of surveillance	Number of health visits per year to each establishment or group of establishments	Number of laboratory examinations per year	Number of molluscs in the sample
Year 1	1	1	150
Year 2	1	1	150
Year 3	1	1	150

SECTION 4
MAINTENANCE OF STATUS FREE FROM INFECTION WITH *BONAMIA OSTREAE*

1. When targeted surveillance is required in order to maintain the status free from infection with *Bonamia ostreae* of a Member State, a zone or a compartment, in accordance with Article 81, all establishments keeping listed species within the Member State, zone or compartment concerned must be subject to health visits and molluscs must be sampled in accordance with Table 5.B, taking into account the risk level of the establishment for the contraction of infection with *Bonamia ostreae*.

2. When determining the frequency of health visits required to maintain the status free from infection with *Bonamia ostreae* of compartments where the health status regarding that disease is dependent on the health status of the aquatic animal populations in surrounding natural waters, the risk for the contraction of infection with *Bonamia ostreae* must be regarded as high.

3. The status free from infection with *Bonamia ostreae* may only be maintained as long as all samples, using the diagnostic methods set out in point 2 of Section 5 have produced negative results for *Bonamia ostreae* and any suspicion of infection with *Bonamia ostreae* has been ruled out in accordance with the diagnostic methods set out in point 3 of Section 5.

Table 5.B

Scheme for Member States, zones or compartments to maintain status free from infection with *Bonamia ostreae*

Risk level(1)	Number of health visits to each establishment/ group of establishments	Number of laboratory examinations	Number of molluscs in the sample
High	1 every year	1 every 2 years	150
Medium	1 every 2 years	1 every 2 years	150
Low	1 every 3 years	1 every 3 years	150

(1) Risk level assigned to the establishment by the competent authority as set out in paragraph 1 of Chapter 2 of Part I other than in the case of dependent compartments where all establishments are deemed to be high risk.

SECTION 5
DIAGNOSTIC AND SAMPLING METHODS

1. The whole animal must be submitted to the laboratory for the performance of the diagnostic tests provided for in points 2 and 3.

2. The diagnostic methods to be used to grant or maintain status free from infection with *Bonamia ostreae,* in accordance with Sections 2 to 4 must follow the detailed diagnostic methods and procedures approved by the EURL for Mollusc Diseases and must be histopathology, tissue imprints or PCR.

3. When a suspicion of infection with *Bonamia ostreae* is required to be confirmed or ruled out in accordance with Article 58, the following visit, sampling and testing procedure must be complied with:

   (a) the investigation must include at least one sampling of 30 molluscs of susceptible species if the suspicion is based on a mortality report, or if not, 150 molluscs of susceptible species after the beginning of the transmission period of *Bonamia ostreae*. When the transmission period is not known, the sampling shall begin in the winter or at the beginning of spring;

   (b) the samples must be tested using the diagnostic methods set out in point (i) following the detailed diagnostic methods and procedures which have been approved by the EURL for Mollusc Diseases:

      (i) the presence of *Bonamia ostreae* must be considered as confirmed when a positive result by histopathology, tissue imprints or *in situ* hybridisation is combined with a positive result by PCR followed by sequencing. If biological material is not available for histopathology, tissue imprints or *in situ* hybridisation, the presence of *Bonamia ostreae* must be considered as confirmed when positive results are obtained using two PCR assays targeting different fragments of the parasite genome and completed by sequencing;

      (ii) the suspicion of the presence of infection with *Bonamia ostreae* must be ruled out, if those tests reveal no further evidence of the presence of *Bonamia ostreae* .

Chapter 6
Eradication, disease-free status and diagnostic methods for infection with white spot syndrome virus (WSSV)

SECTION 1
GENERAL REQUIREMENTS FOR HEALTH VISITS AND SAMPLING

Health visits and sampling for the surveillance referred to in point (b)(ii) of Article 3(2) must comply with the following requirements:

(a) the sampling of crustaceans for laboratory examination must be carried out whenever the water temperature is likely to reach its highest annual point. That requirement concerning water temperature must also apply to health visits where these are feasible;

(b) when farmed crustaceans must be sampled in accordance with the requirements set out in Sections 2 to 4, the following criteria must apply:

  (i) if weak or moribund crustaceans are present in the production units, such crustaceans must primarily be selected. If such crustaceans are not present, those selected must include crustaceans of different size cohorts namely juveniles and adults of the selected susceptible species, proportionally represented in the sample;

  (ii) if more than one water source is utilised for crustacean production, susceptible crustaceans representing all water sources must be included for sampling;

(c) when targeted surveillance in wild populations is required due to the small number of establishments covered by the eradication programme, the number and geographical distribution of the sampling points must be determined to obtain a reasonable coverage of the Member State, zone or compartment. The sampling points must also be representative of the different ecosystems where the wild populations of susceptible species are located namely marine, estuary, river and lake systems. In such situations, the crustaceans to be sampled must be selected as follows:

  (i) in marine and estuary systems areas, one or more of the following species must be selected: *Carcinus maenas, Cancer pagurus, Eriocheir sinensis, Liocarcinus depurator, Liocarcinus puber, Crangon crangon, Homarus gammarus, Palaemon adspersus* or penaeid shrimp species namely *Penaeus japonicus, Penaeus kerathurus, Penaeus semisulcatus*. If those species are not present, the sample must be representative of other susceptible decapod species present;

  (ii) in river and lake systems, one or more of the following species must be selected: *Pacifastacus leniusculus, Astacus leptodactylus, Austropotamobius pallipes* or *Orconectes limosus*. If those species are not present, the sample must be representative of other susceptible decapod species present;

  (iii) if weak or moribund crustaceans are present, such crustaceans must primarily be selected. If such crustaceans are not present, those selected must include crustaceans of different size cohorts namely juveniles and adults of the selected susceptible species, proportionally represented in the sample.

SECTION 2
GRANTING OF THE STATUS FREE FROM INFECTION WITH *WSSV* IN MEMBER STATES, ZONES AND COMPARTMENTS OF UNKNOWN HEALTH STATUS

1. The status free from infection with *WSSV* may only be granted to a Member State, a zone or a compartment with an unknown health status with regard to infection with *WSSV* if all establishments or groups of establishments keeping listed species within the Member State, zone or compartment and where required, sampling points in wild populations, have been subject to the following 2-year scheme:

   (a) the establishments or groups of establishments have been subject to health visits and sampled for a minimum period of 2 consecutive years as laid down in Table 6.A;

   (b) during that 2-year period, the testing of all samples using the diagnostic methods set out in point 2 of Section 5 have produced negative results for infection with WSSV and any suspicion of infection with WSSV has been ruled out in accordance with the diagnostic methods set out in point 3 of Section 5;

2. If infection with WSSV is detected during the 2-year scheme referred to in point 1, before starting a new 2-year scheme, relevant establishments in the Member State, zone or compartment must:

   (a) be subject to the minimum disease control measures laid down in Articles 58 to 65;

   (b) be repopulated with crustaceans from an establishment in a Member State, zone or compartment free from infection with WSSV or from an establishment in a Member State, zone or compartment covered by an eradication programme for that disease.

SECTION 3
GRANTING OF THE STATUS FREE FROM INFECTION WITH WSSV IN MEMBER STATES, ZONES AND COMPARTMENTS KNOWN TO BE INFECTED WITH WSSV

1. The status free from infection with WSSV may only be granted to a Member State, a zone or a compartment known to be infected with WSSV if all establishments keeping listed species within that Member State, zone or compartment have been subject to an eradication programme that complies with the following requirements:

   (a) the minimum control measures laid down in Articles 55 to 65 must have been effectively applied, and a restricted zone of an appropriate size as provided for in point (c) of Article 58(1), where appropriate, divided into a protection zone and surveillance zone; must have been established in the vicinity of the establishment(s) declared infected with WSSV taking into account the requirements set out in point 2;

   (b) all establishments keeping listed species within the protection zone, or where a protection zone has not been established, the restricted zone, not infected with WSSV must be subject to an investigation comprising at least the following:

      (i) the collection of samples for testing of 10 crustaceans, when clinical signs or post-mortem lesions consistent with infection WSSV are

observed, or 150 crustaceans, when clinical signs or post-mortem lesions are not observed; and

(ii) health visits; in those establishments where the tests referred to in (i) have produced negative results, health visits must continue once per month during the season when the water temperature is likely to reach its highest annual points, until the protection zone has been withdrawn in accordance with point (c);

(c) relevant establishments must be emptied in accordance with Articles 62, cleaned disinfected in accordance with Article 63 and fallowed in accordance with Article 64. The duration of the fallowing period must be at least 6 weeks. When all infected establishments are emptied, at least 3 weeks of synchronous fallowing shall be carried out.

When fallowing of the officially declared infected establishments is carried out, the protection zones shall be converted into surveillance zones;

(d) repopulation may only take place when all infected establishments have been emptied, cleaned, disinfected and fallowed in accordance with point (c);

(e) all establishments other than those referred to in point (f) which keep listed species within the Member State, zone or compartment covered by the eradication programme and, when surveillance in wild populations is required, all sampling points selected to provide the greatest coverage of the geographical area included in the eradication programme must be subject at least to the scheme set out in Section 2;

(f) an individual establishment which keeps listed species and which has a health status which is independent of the health status of the surrounding waters is not required to comply with the scheme set out in Section 2 following a disease outbreak provided the establishment complies with the requirements set out in paragraph 3 of Article 80 and is repopulated with crustaceans sourced from Member States, zones or compartments with status free from infection with WSSV.

2. The restricted zone must have been defined on a case-by-case basis taking into account factors influencing the risks for the spread of WSSV to farmed and wild crustaceans, such as:

(i) the number, age, rate and distribution of the mortalities of crustaceans on the establishment or group of establishments infected with WSSV including other establishments and wild crustaceans;

(ii) the distance to and density of neighbouring establishments or groups of establishments including wild crustaceans;

(iii) the proximity to processing establishments, contact establishments or groups of establishments;

(iv) the species, especially susceptible species and vector species, present at the establishments or groups of establishments;

(v) the farming practices applied in the affected and neighbouring establishments and groups of establishments;

(vi) the hydrodynamic conditions; and

(vii) other factors of epidemiological significance identified.

Table 6. A

Scheme for Member States, zones and compartments for the 2-year control period which precedes the achievement of status free from infection with WSSV

Year of surveillance	Number of health visits per year to each establishment or group of establishments	Number of laboratory examinations per year	Number of crustaceans in the sample
Year 1	1	1	150
Year 2	1	1	150

SECTION 4
MAINTENANCE OF STATUS FREE FROM INFECTION WITH *WSSV*

1. When targeted surveillance is required in order to maintain the status free from infection with WSSV of a Member State, a zone or a compartment, in accordance with Article 81, all establishments keeping listed species within the Member State, zone or compartment concerned must be subject to health visits and crustaceans must be sampled in accordance with Table 6.B, taking into account the risk level of the establishment for the contraction of infection with WSSV.

2. In Member States, zones or compartments where the number of establishments is limited and targeted surveillance in those establishments does not provide sufficient epidemiological data, the surveillance to maintain disease-free status must include sampling points selected in accordance with the requirements laid down in point (b) of Section 1.

3. When determining the frequency of health visits required to maintain the status free from infection with WSSV of compartments where the health status regarding that disease is dependent on the health status of the aquatic animal populations in surrounding natural waters, the risk for the contraction of infection with WSSV must be regarded as high.

4. The status free from infection with WSSV may only be maintained as long as all samples, using the diagnostic methods set out in point 2 of Section 5 have produced negative results for WSSV and any suspicion of infection with WSSV has been ruled out in accordance with the diagnostic methods set out in point 3 of Section 5.

Table 6. B

Scheme for Member States, zones or compartments to maintain status free from infection WSSV

Risk level(1)	Number of health visits to each establishment/ group of establishments	Number of laboratory examinations	Number of crustaceans in the sample
High	1 every year	1 every 2 years	150
Medium	1 every 2 years	1 every 2 years	150
Low	1 every 2 years	1 every 4 years	150

(1) Risk level assigned to the establishment by the competent authority as set out in paragraph 1 of Chapter 2 of Part I other than in the case of dependent compartments where all establishments are deemed to be high risk.

SECTION 5
DIAGNOSTIC AND SAMPLING METHODS

1. Samples of integumental epidermis, either dissected or contained within walking legs, pleopods, mouthparts or gills of the test animal must be fixed in 95 % ethanol prior to the preparation of samples for PCR.

   Other samples, fixed for histology and transmission electron microscopy may be collected to support diagnostic data arising from PCR.

2. The diagnostic method and procedures to be used to grant or to maintain disease-free status with regard to infection with WSSV must be PCR followed by sequencing. When applying these diagnostic methods, the corresponding detailed methods and procedures which have been approved by the EURL for Crustacean Diseases must be followed.

   In the case of a positive result from the PCR test, the result must be followed by sequencing of the amplicon before the initial control measures provided for in Article 63 of Regulation (EU) 2016/429 are implemented.

3. When a suspicion of infection with WSSV is required to be confirmed or ruled out in accordance with Article 58, the following visit, sampling and testing procedure must be complied with:

   (a) the investigation must include at least one health visit and one sampling of 10 crustaceans when clinical signs or post-mortem lesions consistent with infection with WSSV are observed or 150 crustaceans when clinical signs or post-mortem lesions are not observed. The samples must be tested using the diagnostic method set out in point 2;

   (b) the presence of WSSV must be considered as confirmed when PCR followed by sequencing, carried out in accordance with the detailed methods and procedures which have been approved by the EURL for Crustacean Diseases test positive for WSSV.

   The suspicion of infection with WSSV may be ruled out, if those tests reveal no further evidence of the presence of the virus.

PART III
REQUIREMENTS FOR DEMONSTRATING THE IMPLEMENTATION OF SURVEILLANCE PROGRAMMES FOR CATEGORY C DISEASES AND FOR RESTARTING THOSE PROGRAMMES AFTER A DISEASE OUTBREAK

Part III covers the requirements for establishments to demonstrate the implementation of a surveillance programme for a particular disease and the requirements to restart that surveillance programme following a disease outbreak.

Viral haemorrhagic septicaemia (VHS)	Chapter 1
Infectious haematopoietic necrosis (IHN)	Chapter 1
Infection with HPR-deleted infectious salmon anaemia virus	Chapter 2
Infection with *Marteilia refringens*	Chapter 3
Infection with *Bonamia exitiosa*	Chapter 4
Infection with *Bonamia ostreae*	Chapter 5
Infection with white spot syndrome virus (WSSV)	Chapter 6

Chapter 1
Requirements for establishments to demonstrate the implementation of a surveillance programme for VHS or IHN and requirements to re-start that programme following a disease outbreak

SECTION 1
GENERAL REQUIREMENTS FOR HEALTH VISITS AND SAMPLING FOR VHS AND IHN

The health visits and sampling referred to in point (b)(iv) of Article 3(2) must comply with the following requirements:

(a) health visits and sampling must be carried out during the period of the year when the water temperature is below 14°C or when temperatures below 14°C are not reached, samples must be taken at the lowest annual points;

(b) all production units, such as ponds, tanks and net cages, must be examined for the presence of dead, weak or abnormally behaving fish. Particular attention must be paid to the water outlet area where weak fish tend to accumulate because of the water current;

(c) fish of listed species to be collected as samples must be selected as follows:

(i) if rainbow trout are present, only fish of that species must be selected for sampling, except where other susceptible species are present which show typical signs of VHS or IHN; if rainbow trout are not present, the sample must be representative of all other susceptible species which are present;

(ii) if weak, abnormally behaving or freshly dead but not decomposed fish are present, such fish must be selected; if more than one water source is utilised for fish production, fish representing all water sources must be included in the sample;

(iii) the fish selected must include fish collected in such a way that all parts of the establishment, as well as all year classes, are proportionally represented in the sample.

SECTION 2

SPECIFIC REQUIREMENTS TO DEMONSTRATE THE IMPLEMENTATION OF A SURVEILLANCE PROGRAMME

1. Health visits must be carried out and fish must be sampled in accordance with Section 1 and Table 1.

2. Samples which are collected in accordance with Section 1 and Table 1 must be tested using the diagnostic methods set out in point 2 of Section 5 of Chapter 1 of Part II and produce negative results for VHS or IHN.

SECTION 3

REQUIREMENTS TO RE-START A SURVEILLANCE PROGRAMME AFTER A DISEASE OUTBREAK

An establishment which has been infected with VHS or IHN, may restart a surveillance programme for these diseases provided that:

(a) it has been emptied in accordance with Article 62, cleaned and disinfected in accordance with Article 63, and fallowed in accordance with Article 64; and

(b) repopulation occurs using fish that originate from establishments which are:

(i) in a Member State, a zone or a compartment free from VHS or IHN;

(ii) in a Member State, a zone or a compartment covered by an eradication programme for VHS or IHN; or

(iii) implementing a surveillance programme for VHS or IHN.

Table 1

Surveillance programme for VHS/IHN

Risk level(1)	**Number of health visits per year to each establishment**	**Number of fish in the sample**(2)
High	1 every year	30
Medium	1 every 2 years	30
Low	1 every 3 years	30

(1) In the case of coastal zones or coastal compartments, the samples must be collected no sooner than 3 weeks after the transfer of the fish from fresh to saltwater.

(2) Risk level assigned to the establishment by the competent authority as set out in paragraph 1 of Chapter 2 of Part I.Maximum number of fish per pool: 10

Chapter 2
Requirements for establishments to demonstrate the implementation of a surveillance programme for HPR-deleted ISAV and to re-start that programme after a disease outbreak

SECTION 1
GENERAL REQUIREMENTS FOR HEALTH VISITS AND SAMPLING FOR INFECTION WITH HPR-DELETED ISAV

The health visits and sampling referred to in point (b)(iv) of Article 3(2) must comply with the following requirements:

(a) health visits and sampling must take into account all production units, such as ponds, tanks and net cages, to determine if dead, weak or abnormally behaving fish are present. Particular attention must be paid to the edge of cages or the water outlet area as relevant, where weak fish tend to accumulate because of the water current;

(b) the fish to be collected as samples must be selected as follows:

(i) only moribund or freshly dead but not decomposed fish must be selected; in particular fish demonstrating anaemia, bleeding or other clinical signs suggesting circulatory disturbances must be prioritised for collection;

(ii) if Atlantic salmon are present, only fish of that species must be selected for sampling, except where other susceptible species are present which show typical signs of ISA. If there are no Atlantic salmon in the establishment, other listed species must be sampled;

(iii) if more than one water source is utilised for fish production, fish representing all water sources must be included in the sample;

(iv) the fish selected must include fish collected in such a way that all production units, such as net cages, tanks and ponds, as well as all year classes in the establishment are proportionally represented in the sample.

SECTION 2

SPECIFIC REQUIREMENTS TO DEMONSTRATE THE IMPLEMENTATION OF A SURVEILLANCE PROGRAMME

1. Health visits must be carried out and fish must be sampled in accordance with Section 1 and Table 2.

2. Samples which are collected in accordance with Section 1 and Table 2 must be tested using the diagnostic methods set out in point 2 of Section 5 of Chapter 2 of Part II and produce negative results for HPR-deleted ISAV.

Table 2

Surveillance programme for HPR-deleted ISAV

Risk level(1)	**Number of health visits per year to each establishment**	**Number of laboratory examinations per year**	**Number of fish in the sample**
High	2	2(2)	30
Medium	1	1(3)	30
Low	1 every 2 years	1 every two years	30
Maximum number of fish per pool: 5			

(1) Risk level assigned to the establishment by the competent authority as set out in paragraph 1 of Chapter 2 of Part I

(2) Samples must be collected during spring and autumn when two samples are required each year

(3) Samples must be collected during spring or autumn when only one sample is required per year

SECTION 3

REQUIREMENTS TO RE-START A SURVEILLANCE PROGRAMME AFTER A DISEASE OUTBREAK

An establishment which has been infected with HPR-deleted ISAV may restart a surveillance programme for that diseases provided that:

(a) it has been emptied in accordance with Article 62, cleaned and disinfected in accordance with Article 63, and fallowed in accordance with Article 64; and

(b) repopulation occurs using fish that originate from establishments which are:

  (i) in a Member State, a zone or a compartment free from infection with HPR-deleted ISAV;

  (ii) in a Member State, a zone or a compartment covered by an eradication programme for infection with HPR-deleted ISAV; or

  (iii) implementing a surveillance programme for infection with HPR-deleted ISAV.

Chapter 3
Requirements for establishments to demonstrate the implementation of a surveillance programme for infection with *Marteilia refringens* and requirements to re-start that programme following a disease outbreak

SECTION 1
GENERAL REQUIREMENTS FOR HEALTH VISITS AND SAMPLING FOR INFECTION WITH *MARTEILIA REFRINGENS*

The health visits and sampling referred to in point (b)(iv) of Article 3(2) must comply with the following requirements:

(a) health visits and sampling for laboratory examination must be carried out in the period of the year when prevalence of the parasite in the Member State, zone or compartment is known to be maximal. When such data is not available, sampling shall be carried out just after the water temperature has exceeded 17 °C;

(b) when molluscs are to be sampled in accordance with the requirements set out in Table 3, the following criteria must apply:

  (i) *Ostrea spp*. must be sampled. If *Ostrea* spp. are not present, the sample must be representative of all other listed species present;

  (ii) if weak, gaping or freshly dead but not decomposed molluscs are present in the production units, such molluscs must primarily be selected. If such molluscs are not present, the molluscs selected must include the oldest healthy molluscs;

  (iii) when sampling in mollusc establishments which utilise more than one water source for mollusc production, molluscs representing all water sources must be included for sampling in such a way that all parts of the establishment are proportionally represented in the sample;

  (iv) when sampling in mollusc establishments or groups of establishments, molluscs from a sufficient number of sampling points must be included in the sample in such a way that all parts of the establishment or group of establishments are proportionally represented in the sample. The main factors to be considered for the selection of those sampling points are stocking density, water flows, the presence of susceptible species, the presence of vector species, bathymetry and management practices. Natural beds within or adjacent to the establishment or group of establishments must be included in the sampling.

SECTION 2
SPECIFIC REQUIREMENTS TO DEMONSTRATE THE IMPLEMENTATION OF A SURVEILLANCE PROGRAMME

1. Health visits must be carried out and molluscs must be sampled in accordance with Section 1 and Table 3.

2. Samples which are collected in accordance with Section 1 and Table 3 must be tested using the diagnostic methods set out in point 2 of Section 5 of Chapter 3 of Part II and produce negative results for *Marteilia refringens*.

Table 3

Surveillance programme for *Marteilia refringens*

Risk level(1)	**Number of health visits to each establishment/ group of establishments**	**Number of laboratory examinations**	**Number of molluscs in the sample**
High	1 every year	1 every 2 years	150
Medium	1 every 2 years	1 every 2 years	150
Low	1 every 2 years	1 every 4 years	150

(1) Risk level assigned to the establishment by the competent authority as set out in paragraph 1 of Chapter 2 of Part I

SECTION 3
REQUIREMENTS TO RE-START A SURVEILLANCE PROGRAMME AFTER A DISEASE OUTBREAK

An establishment which has been infected with *Marteilia refringens* may re-start a surveillance programme for that disease provided that:

(a) it has been emptied in accordance with Article 62, cleaned and disinfected in accordance with Article 63, and fallowed in accordance with Article 64; and

(b) repopulation occurs using fish that originate from establishments which are:

(i) in a Member State, a zone or a compartment free from infection with *Marteilia refringens*;

(ii) in a Member State, a zone or a compartment covered by an eradication programme for infection with *Marteilia refringens*; or

(iii) implementing a surveillance programme for infection with *Marteilia refringens.*

Chapter 4
Requirements for establishments to demonstrate the implementation of a surveillance programme for infection with *Bonamia exitiosa* and to re-start that programme following a disease outbreak

SECTION 1
GENERAL REQUIREMENTS FOR HEALTH VISITS AND SAMPLING FOR INFECTION WITH *BONAMIA EXITIOSA*

The health visits and sampling referred to in point (b)(iv) of Article 3(2) must comply with the following requirements:

(a) health visits and sampling of production units must be carried out in the period of the year when prevalence of *Bonamia exitiosa* in the Member State, zone or compartment is known to be maximal. When such data is not available, sampling shall be carried out twice a year, in spring and autumn;

(b) when molluscs are sampled in accordance with the requirements set out in Table 4, the following criteria must apply:

(i) if *Ostrea* spp. are present, only oysters of that species must be selected for sampling. If *Ostrea spp*. are not present, the sample must be representative of all other susceptible species present;

(ii) if weak, gaping or freshly dead but not decomposed molluscs are present, such molluscs must primarily be selected. If such molluscs are not present, the molluscs selected must include the oldest healthy molluscs;

(iii) when sampling in establishments which utilise more than one water source for mollusc production, molluscs representing all water sources must be included for sampling in such a way that all parts of the establishment are proportionally represented in the sample;

(iv) when sampling in establishments or groups of establishments, molluscs from a sufficient number of sampling points must be included in the sample in such a way that all parts of the establishment or group of establishments are proportionally represented in the sample. The main factors to be considered for the selection of those sampling points are stocking density, water flows, the presence of susceptible species, the presence of vector species (e.g.*Crassostrea gigas*), bathymetry and management practices. Natural beds within or adjacent to the establishment or group of establishments must be included in the sampling.

SECTION 2

SPECIFIC REQUIREMENTS TO DEMONSTRATE THE IMPLEMENTATION OF A SURVEILLANCE PROGRAMME

1. Health visits must be carried out and molluscs must be sampled in accordance with Section 1 and Table 4.

2. Samples which are collected in accordance with Section 1 and Table 4 must be tested using the diagnostic methods referred to in point 2 of Section 5 of Chapter 4 of Part II and produce negative results for *Bonamia exitiosa*.

Table 4

Surveillance programme for infection with *Bonamia exitiosa*

Risk level(1)	Number of health visits to each establishment/ group of establishments	Number of laboratory examinations	Number of molluscs in the sample
High	1 every year	1 every 2 years	150
Medium	1 every 2 years	1 every 2 years	150
Low	1 every 2 years	1 every 4 years	150

(1) Risk level assigned to the establishment by the competent authority as set out in paragraph 1 of Chapter 2 of Part I.

SECTION 3
REQUIREMENTS TO RE-START A SURVEILLANCE PROGRAMME AFTER A DISEASE OUTBREAK

An establishment which has been infected with *Bonamia exitiosa* may re-start a surveillance programme provided that:

(a) it has been emptied in accordance with Article 62, cleaned and disinfected in accordance with Article 63, and fallowed in accordance with Article 64; and

(b) repopulation occurs using fish that originate from establishments which are:

(i) in a Member State, a zone or a compartment free from infection with *Bonamia exitiosa*;

(ii) in a Member State, a zone or a compartment covered by an eradication programme for infection with *Bonamia exitiosa*; or

(iii) implementing a surveillance programme for infection with *Bonamia exitiosa.*

Chapter 5
Requirements for establishments to demonstrate the implementation of a surveillance programme for infection with *Bonamia ostreae* and to re-start that programme following a disease outbreak

SECTION 1
GENERAL REQUIREMENTS FOR HEALTH VISITS AND SAMPLING FOR INFECTION WITH *BONAMIA OSTREAE*

The health visits and sampling referred to in point (b)(iv) of Article 3(2) must comply with the following requirements:

(a) health visits and sampling of production units shall be carried out in the period of the year when prevalence of *Bonamia ostreae* in the Member State, zone or compartment is known to be maximal. When such data is not available, sampling shall be carried out in winter or at the beginning of spring;

(b) when molluscs are to be sampled in accordance with the requirements set out in Table 5, the following criteria must apply:

(i) if *Ostrea edulis* are present, only oysters of that species must be selected for sampling. If *Ostrea edulis* are not present, the sample must be representative of all other susceptible species present;

(ii) if weak, gaping or freshly dead but not decomposed molluscs are present, such molluscs must primarily be selected. If such molluscs are not present, the molluscs selected must include the oldest healthy molluscs;

(iii) when sampling in establishments which utilise more than one water source for mollusc production, molluscs representing all water sources must be included for sampling in such a way that all parts of the establishment are proportionally represented in the sample;

(iv) when sampling in mollusc establishments or groups of establishments, molluscs from a sufficient number of sampling points must be included in the sample. The main factors to be considered for the selection of those sampling points are stocking density, water flows, the presence of susceptible species, the presence of vector species, bathymetry and management practices. Natural beds within or adjacent to the establishment or group of establishments must be included in the sampling.

SECTION 2

SPECIFIC REQUIREMENTS TO DEMONSTRATE THE IMPLEMENTATION OF A SURVEILLANCE PROGRAMME

1. Health visits must be carried out and molluscs must be sampled in accordance with Section 1 and Table 5.

2. Samples which are collected in accordance with Section 1 and Table 5 must be tested using the diagnostic methods referred to in point 2 of Section 5 of Chapter 5 of Part II and produce negative results for *Bonamia ostreae*.

Table 5

Surveillance programme for infection with *Bonamia ostreae*

Risk level(1)	**Number of health visits to each establishment/ group of establishments**	**Number of laboratory examinations**	**Number of molluscs in the sample**
High	1 every year	1 every 2 years	150
Medium	1 every 2 years	1 every 2 years	150
Low	1 every 2 years	1 every 4 years	150

(1) Risk level assigned to the establishment by the competent authority as set out in paragraph 1 of Chapter 2 of Part I

SECTION 3

REQUIREMENTS TO RE-START A SURVEILLANCE PROGRAMME AFTER A DISEASE OUTBREAK

An establishment which has been infected with *Bonamia ostreae* may re-start the surveillance programme for that disease provided that:

(a) it has been emptied in accordance with Article 62, cleaned and disinfected in accordance with Article 63, and fallowed in accordance with Article 64; and

(b) repopulation occurs using fish that originate from establishments which are:

(i) in a Member State, a zone or a compartment free from infection with *Bonamia ostreae*;

(ii) in a Member State, a zone or a compartment covered by an eradication programme for infection with *Bonamia ostreae*; or

(iii) implementing a surveillance programme for infection with *Bonamia ostreae.*

Chapter 6
Requirements for establishments to demonstrate the implementation of a surveillance programme for infection with WSSV and to re-start that programme following a disease outbreak

SECTION 1
GENERAL REQUIREMENTS FOR HEALTH VISITS AND SAMPLING FOR INFECTION WITH INFECTION WITH WSSV

The health visits and sampling referred to in point (b)(iv) of Article 3(2) must comply with the following requirements:

(a) the sampling of crustaceans for laboratory examination must be carried out whenever the water temperature is likely to reach its highest annual point. That requirement concerning water temperature must also apply to health visits where these are feasible and appropriate;

(b) when farmed crustaceans are to be sampled in accordance with the requirements set out in Table 6, the following criteria must apply:

(i) if weak or moribund crustaceans are present in the production units, such crustaceans must primarily be selected. If such crustaceans are not present, those selected must include crustaceans of different size cohorts namely juveniles and adults, of the selected susceptible species, proportionally represented in the sample;

(ii) if more than one water source is utilised for crustacean production, susceptible crustaceans representing all water sources must be included for sampling.

SECTION 2
SPECIFIC REQUIREMENTS TO DEMONSTRATE THE IMPLEMENTATION OF A SURVEILLANCE PROGRAMME

1. Health visits shall be carried out and crustaceans shall be sampled in accordance with Section 1 and Table 6.

2. Samples which are collected in accordance with Section 1 and Table 6 must be tested using the diagnostic methods referred to in point 2 of Section 5 of Chapter 6 of Part II and produce negative results for infection with WSSV.

Table 6

Surveillance programme for infection with WSSV

Risk level(1)	Number of health visits to each establishment/ group of establishments	Number of laboratory examinations	Number of crustaceans in the sample
High	1 every year	1 every 2 years	150
Medium	1 every 2 years	1 every 2 years	150
Low	1 every 2 years	1 every 4 years	150

(1) Risk level assigned to the establishment by the competent authority as set out in paragraph 1 of Chapter 2 of Part I

SECTION 3
REQUIREMENTS TO RE-START A SURVEILLANCE PROGRAMME AFTER A DISEASE OUTBREAK

An establishment which has been infected with WSSV may re-start a surveillance programme for that disease provided that:

(a) it has been emptied in accordance with Article 62, cleaned and disinfected in accordance with Article 63, and fallowed in accordance with Article 64; and

(b) repopulation occurs using fish that originate from establishments which are:

(i) in a Member State, a zone or a compartment free from infection with WSSV;

(ii) in a Member State, a zone or a compartment covered by an eradication programme for infection with WSSV; or

(iii) implementing a surveillance programme for infection with WSSV.