CHAPTER 1.4.

AQUATIC ANIMAL DISEASE SURVEILLANCE

Article 1.4.1.

Purpose

This chapter provides guidance on the *surveillance* approaches to be used by a *Competent Authority* to make and maintain a *self-declaration of freedom from disease* or to confirm the occurrence of a *listed disease* or an *emerging disease*.

Article 1.4.2.

Introduction and scope

This chapter supports a *Competent Authority* to meet the requirements for *self-declaration of freedom from disease* at the level of a country, *zone* or *compartment*, and for maintenance of freedom, that are presented in each disease-specific chapter. It also provides a *Competent Authority* with guidance to meet the requirements of *notification* of a *listed disease* or an *emerging disease* in accordance with Chapter 1.1.

This chapter is not intended to provide detailed technical guidance on *surveillance* design or analysis. *Competent Authorities* are encouraged to consult published literature and seek appropriate expertise to design and analyse *surveillance* programmes that meet the requirements of the *Aquatic Code*.

1) The general requirements of a *surveillance* system necessary to support a *self-declaration of freedom from disease* are specified in Articles 1.4.5. to 1.4.8.
2) The criteria that have been used to set the periods specified in each disease-specific chapter for *basic biosecurity conditions* to be in place, or for *targeted surveillance* that should be undertaken, prior to claiming freedom, are included in Articles 1.4.9. and 1.4.10.
3) The requirements for each of the four pathways for claiming freedom, and for maintaining freedom, are introduced in Article 1.4.3. and described in detail in Articles 1.4.11. to 1.4.15.
4) Guidance on the design of surveys to demonstrate freedom from *disease*, and for combining multiple sources of *surveillance* information are provided in Articles 1.4.16. and Article 1.4.17., respectively.
5) Article 1.4.18. provides guidance on diagnostic confirmation of *listed diseases* or an *emerging disease*.

Competent Authorities should refer to the relevant disease-specific chapter of the *Aquatic Manual* for recommendations on sample collection and appropriate diagnostic methods for *surveillance* and *diagnosis* of *listed diseases*. The relevant disease-specific chapter of the *Aquatic Manual* should also be consulted for the necessary information on epidemiology and diagnostic performance of assays required for *surveillance* programme design.

Article 1.4.3.

Pathways for demonstrating freedom from disease

Competent Authorities may use one of four pathways to make a *self-declaration of freedom from disease*. Each pathway outlines the *aquatic animal* health circumstances and requirements that should be met for a self-declaration to be made. Any one of these four pathways may be utilised; however, a *Competent Authority* should provide evidence that all relevant requirements to demonstrate *disease* freedom have been met as described in this chapter and the relevant disease-specific chapter of the *Aquatic Code* including when water bodies are shared with other countries or are under the control of different *Competent Authorities*. The four pathways are:

1. Absence of susceptible species

   This pathway may be utilised if, as described in Article 1.4.11., it can be demonstrated that no *susceptible species* are present at the country or *zone* level.

2. Historical freedom

This pathway may be utilised if, as described in Article 1.4.12., there is evidence of historical absence of a *disease* at the country or *zone* level, that is supported primarily by *passive surveillance* information generated by a country's *early detection system*. *Targeted surveillance* data may also be used in this pathway, where appropriate.

3. Targeted surveillance

This pathway may be utilised at the country, *zone* or *compartment* level. The pathway primarily uses *targeted surveillance* data, but other sources of evidence may be utilised as described in Article 1.4.13. *Passive surveillance* information may also be used in this pathway, where appropriate.

4. Returning to freedom

This pathway may be utilised, as described in Article 1.4.14., in circumstances where a self-declaration had been made, but free status was subsequently lost due to detection of the *disease* for a country, *zone* or *compartment*.

Table 1.1. A summary of the four pathways for *self-declaration of freedom from disease*, including the types of primary and secondary *surveillance* information, and the applicable level of application for either a country, *zone* or *compartment*.

Pathway	Primary surveillance evidence to claim disease freedom	Secondary evidence to claim freedom (if required)	Applicable level of application
1. Absence of *susceptible species*	Surveys, historical data, import records, environmental information	None	Country, *zone*
2. Historical freedom	*Passive surveillance*	*Targeted surveillance* (in populations where *passive surveillance* is not appropriate)	Country, *zone*
3. *Targeted surveillance*	*Targeted surveillance*	*Passive surveillance* (in appropriate populations)	Country, *zone*, *compartment*
4. Returning to freedom	*Targeted surveillance*	*Passive surveillance* (in appropriate populations)	Country, *zone*, *compartment*

Article 1.4.4.

Publication by WOAH of a self-declaration of freedom from disease by a Member Country

A Member Country may make a *self-declaration of freedom from disease* in a country, *zone* or *compartment*. The Member Country should inform WOAH of the claimed status for a country, *zone* or *compartment* and WOAH may publish the self-declaration.

A Member Country requesting the publication of a self-declaration should follow the Standard Operating Procedure (available on the WOAH website) for submission and provide documented information on its compliance with the relevant chapters of the *Aquatic Code*. This information should include, but is not limited to the following:

1) the scope of the declaration, i.e. the specific *disease*, the level of freedom (country, *zone* or *compartment*) and the pathway utilised to claim or return to *disease* freedom;
2) information to verify that *basic biosecurity conditions* and the requirements of *surveillance* systems have been met;
3) details of the *surveillance* design and assumptions;
4) the *surveillance* analysis and results;
5) the measures implemented to maintain freedom.

The *self-declaration of freedom* will be published only after all the information provided has been received and administrative and technical screening has been performed by WOAH, with a satisfactory outcome. Publication does not however, imply endorsement of the claim of freedom by WOAH and does not reflect the official opinion of WOAH. Responsibility for the accuracy of the information contained in a self-declaration lies entirely with the WOAH Delegate of the Member Country concerned.

An *outbreak* in a Member Country, a *zone* or a *compartment* having a self-declared free status results in the loss of the self-declared free status. The notification of an *outbreak* in a country, *zone* or *compartment* for which a *self-declaration of freedom* has been made, will result in an update of the WOAH website concerning the original declaration. A Member

Country wishing to reclaim a lost free status should submit a new self-declaration following the procedure described in this chapter.

Article 1.4.5.

Biosecurity and surveillance system requirements

The following *biosecurity* and *surveillance* system requirements should be met for any *self-declaration of freedom from disease* in the given country, *zone* or *compartment*:

1) the quality of *Aquatic Animal Health Services* can be substantiated to meet the requirements of Chapter 3.1.;
2) *basic biosecurity conditions* (which include an *early detection system*) as described in Article 1.4.6. are in place;
3) there has been no vaccination of susceptible *aquatic animals* for the specific *disease* from the implementation of the *basic biosecurity conditions* prior to self-declaration;
4) the *Aquatic Animal Health Services* have sufficient capacity and expertise to investigate and report *disease* events to a *Competent Authority*;
5) a *Competent Authority* has access to appropriate diagnostic capability (from a laboratory with a quality management system that meets requirements of Chapter 1.1.1. of the *Aquatic Manual*) to confirm or exclude cases of *listed diseases* and *emerging diseases* in accordance with Article 1.4.18.

Article 1.4.6.

Basic biosecurity conditions

Basic biosecurity conditions include requirements for preventing the introduction and spread of a specific *disease* and for detection of the *disease* should it occur. The requirements for *basic biosecurity conditions* include:

1) an *early detection system* (as described in Article 1.4.7.);
2) measures to prevent the introduction of the *pathogenic agent* into a country, *zone* or *compartment*, or the spread within or from *infected zones* and *protection zones*, in accordance with the relevant disease-specific chapter.

In making a self-declaration of freedom from a specific *disease* for a country, *zone* or *compartment*, a *Competent Authority* should describe how all of the requirements for *basic biosecurity conditions* relevant to its declaration, are continuously met.

Article 1.4.7.

Early detection system

The *early detection system* of a *Competent Authority* is important to generate evidence for claims of *disease* freedom and to provide assurance that a change in *disease* status would be rapidly discovered.

A *self-declaration of freedom from disease* needs to document that the *early detection system* fulfils each of the requirements below:

1) observers (e.g. the personnel of *aquaculture establishments*, processors, transportation services) have broad awareness of the characteristic signs of *listed diseases* and *emerging diseases*;
2) *veterinarians* and *aquatic animal health professionals* are trained in recognising and reporting suspicion of *listed disease* and *emerging disease* occurrence;
3) the *Aquatic Animal Health Services* have capacity to undertake rapid and effective *disease* investigation based on a national chain of command led by a *Competent Authority*;
4) the *Aquatic Animal Health Services* have access to sufficient diagnostic capability (from a laboratory with a quality management system that meets requirements of Chapter 1.1.1. of the *Aquatic Manual*) to confirm or exclude cases of *listed diseases* and the capacity and expertise to investigate *emerging diseases* as described in Article 1.4.18.;
5) *veterinarians*, *aquatic animal health professionals* and others with an occupational role with *aquatic animals* have a legal obligation to report suspicion of the occurrence of *listed diseases* or *emerging diseases* to a *Competent Authority*.

The sensitivity of an *early detection system* is the likelihood that the *disease* will be detected if present. Of fundamental importance is *disease* reporting by farmers, *aquatic animal health professionals*, *veterinarians* and others to initiate the necessary steps of *passive surveillance*. Specifically, a *Competent Authority* should be able to demonstrate that efforts

have been made to make relevant observers (e.g. farmers and fishers) aware of signs of *listed diseases* and *emerging diseases*, and secondly the obligation of farmers, *aquatic animal health professionals*, *veterinarians* and others with an occupational role with *aquatic animals* to report suspicion. The underpinning legal instruments should be cited.

The capacity of the *Aquatic Animal Health Services* to respond to suspicion of a *listed disease* can be evidenced by response plans, and a descriptive chain of command that will result in an official declaration that the *pathogenic agent* has been detected. Standard operating procedures for diagnostic assays for *listed diseases* and accreditation to internationally recognised laboratory standards can demonstrate the capacity of the *Aquatic Animal Health Services* to detect *listed diseases*. In addition, the effective functioning of the *early detection system* is best illustrated through examples of investigations in response to reported suspicion of *disease*. The sensitivity of an *early detection system* (i.e. the likelihood of *pathogenic agent* detection following introduction) can be quantified, for example, by use of a scenario tree model; however, in most circumstances a qualitative assessment will be sufficient.

Article 1.4.8.

Requirements for passive surveillance

In addition to the characteristics of an *early detection system* described in Article 1.4.7., the conditions described in this article should be met for *passive surveillance* information to be utilised for a *self-declaration of freedom from disease*.

1) The conditions, which apply to each defined *study population* of *susceptible species* of a specific *disease*, are that:

    a) conditions (biotic and abiotic) are conducive to clinical expression of the *infection*, such that if the *pathogenic agent* were present within the population of *susceptible species*, it would produce signs of the *disease* at least seasonally;

    b) observation of signs of the *disease*, which may include increased mortality, would lead to investigation and, where appropriate, reporting to a *Competent Authority*;

    c) populations of susceptible farmed *aquatic animals* should be under sufficient observation, such that, if signs of the *disease* were to occur, they would be observed;

    d) for populations of susceptible wild *aquatic animals*, they should:

        i) be under sufficient observation, such that if signs of the *disease* were to occur, they would be observed and reported, or

        ii) be epidemiologically linked to farmed populations, such that if the *disease* were to occur in wild *aquatic animal* populations it would be observed and reported in adjacent farmed populations.

2) *Passive surveillance* depends primarily on observers (e.g. farmers, *aquatic animal health professionals*, *veterinarians* and others) recognizing signs of *disease* that are suspicious of a *listed disease* or unexplained increased mortality and reporting them to a *Competent Authority*. For wild populations, the requirements of points 1a), b) and d) may not be met under most circumstances and, therefore, *passive surveillance* will be insufficiently sensitive. If a *Competent Authority* utilises *passive surveillance* information for defined populations of wild *aquatic animals*, it should demonstrate that the conditions of this article have been met, and that the *early detection system* will result in detection of the *disease* should it occur.

3) Awareness of signs of *disease* and the necessary level of observation is best demonstrated through examples of reporting by farmers, *aquatic animal health professionals*, *veterinarians* and others to a *Competent Authority*. In addition to reporting, information for *passive surveillance* may originate from inspections at processing plants, routine visits by government officials and surveys (e.g. fisheries and aquatic fauna surveys), submissions to laboratories, *aquaculture establishment* records (e.g. mortality, medicine use, etc.).

4) Evidence from published literature will generally be sufficient to demonstrate the environmental conditions in which *infection* of *susceptible species* will result in clinical signs. This information should be supplemented with data on the environmental conditions for the *target populations*.

5) *Passive surveillance* only contributes to the *early detection system* if observations and investigations that lead to suspicion of *listed diseases* or *emerging diseases* are rapidly reported, to allow a *Competent Authority* to undertake their own investigation.

Article 1.4.9.

Required periods for basic biosecurity conditions

1) Prior to a Member Country making a *self-declaration of freedom from disease*, *basic biosecurity conditions* should be in place for a sufficient duration, so that, by the end of the period, should the *disease* have been introduced before the *basic biosecurity conditions* began:
   a) the specific *pathogenic agent* would not remain present in the environment (see pathway 1 – absence of *susceptible species*); or
   b) the *disease* would manifest clinically and be detected by the country's *early detection system*(see pathway 2 – historical freedom); or
   c) by the time t*argeted surveillance* commenced (see pathway 3 – T*argeted surveillance*), *infection* levels would have reached the minimum *prevalence* estimate (i.e. the design *prevalence*) used in the survey design to calculate the sample sizes (e.g. number of *aquaculture establishments* and *aquatic animals* needed to demonstrate freedom).
2) Each disease-specific chapter of the *Aquatic Code* includes minimum periods that *basic biosecurity conditions* should be in place prior to a *self-declaration of freedom*. These periods reference a default minimum period or a longer period if determined necessary based on the factors described below:
   a) For pathway 1, the default minimum period of *basic biosecurity conditions* required prior to a self-declaration, for all *listed diseases*, is six months. It is expected that this period will be sufficient for most *diseases* to ensure that no viable *pathogenic agent* introduced via *aquatic animal commodities* has remained present in the environment, and the *early detection system* was well established and demonstrated to be functioning. The required period that *basic biosecurity conditions* should be in place prior to making a self-declaration, using this pathway, is determined for each *listed disease* based on its epidemiology (e.g. agent stability in the environment, presence of resistant life stages, *vectors*), and a period longer than the default minimum may be specified in the relevant disease-specific chapter of the *Aquatic Code*.
   b) For pathway 2, the default minimum period of *basic biosecurity conditions* required prior to a self-declaration, for all *listed diseases*, is ten years. This period is the minimum required to achieve 95% likelihood of freedom if the annual likelihood of detection is approximately 30%. However, if the average annual likelihood of detection is considered to be less than 30% (following consideration of the factors below), the minimum period required for *basic biosecurity conditions* defined in the relevant disease-specific chapter of the *Aquatic Code* will be set to a period longer than ten years, as appropriate. An evaluation of the following factors will determine whether a period longer than ten years is recommended in the disease-specific chapters:
      i) the maximum duration of the production cycle for the *susceptible species*;
      ii) the life stages at which *aquatic animals* are susceptible;
      iii) the variation in predilection to clinical *disease* among *susceptible species*;
      iv) the expected severity and duration of clinical signs in the *susceptible species*;
      v) environmental conditions that influence levels of *infection* and clinical expression, including seasonality of the *disease* (i.e. periods of the year when *prevalence* and intensity of *infection* are highest and most conducive to detection);
      vi) factors specific to the *pathogenic agent* (e.g. production of spores);
      vii) production systems and management practices that would affect observation of clinical signs if they were to occur;
      viii) any other relevant factors that may influence presentation of clinical signs and observation of the *disease* should it be present.
   c) For pathway 3, the default minimum period of *basic biosecurity conditions* required prior to commencement of *targeted surveillance* will be one year. It is expected that this period will be sufficient under most circumstances for a *disease* to reach a *prevalence* sufficiently high to be detected by a survey designed in accordance with the recommendations of this chapter. However, the epidemiology of a *disease* and nature of production systems may limit the increase in *prevalence* and intensity of *infection* in the *susceptible species* following introduction of the *disease*. In these instances, the minimum period required for *basic biosecurity conditions* defined in the relevant disease-specific chapter of the *Aquatic Code* will be set to a period longer than one year, as appropriate. An evaluation of the following factors will determine whether a period longer than one year is required:
      i) the maximum duration of the production cycle for the *susceptible species*;
      ii) the life stages at which *aquatic animals* are susceptible;
      iii) seasonality of the *disease* (periods of the year when *prevalence* and intensity of *infection* is highest and most conducive to detection);

iv) production systems and management practices that would affect occurrence of *infection*;

v) any other relevant factors that may influence the expected rate of increase in *prevalence* and intensity of *infection* in *susceptible species* following introduction of the *disease*.

d) Pathway 4 is only applicable following the loss of *disease* freedom due to a *disease outbreak*. This circumstance implies a failure of *basic biosecurity conditions* to prevent the introduction of the *disease*. The pathway of *disease* introduction should be investigated and *basic biosecurity conditions* should be reviewed and modified as necessary to reduce the likelihood of *disease* introduction by the same or similar routes. Mitigation measures should be implemented following eradication of the *disease*, and prior to commencement of any *targeted surveillance* that will be utilised as evidence for a subsequent self-declaration.

Article 1.4.10.

Required periods for targeted surveillance

Prior to a *Competent Authority* making a *self-declaration of freedom from disease* utilising pathway 3 or pathway 4, *targeted surveillance* should be conducted for a defined period, as described in the relevant disease-specific chapter of the *Aquatic Code*. The period of *targeted surveillance* is determined for each disease-specific chapter of the *Aquatic Code*, based on the factors described below:

1) the maximum duration of the production cycle for the *susceptible species*;

2) the life stages at which *aquatic animals* are susceptible;

3) seasonality of the *disease* (periods of the year when *prevalence* and intensity of *infection* is highest and most conducive to detection);

4) production systems and management practices that would affect the seasonal occurrence of *infection*.

For a country or *zone*, the minimum default period for which *targeted surveillance* should occur prior to a *self-declaration of freedom* is two years. During the period of *targeted surveillance*, surveys should occur during defined time periods when conditions are optimal for detection of the *pathogenic agent* (e.g. seasons, temperatures, and life stages). All populations of *susceptible species* in the country or *zone* should be considered in the design of each survey (i.e. included in the sampling frame). Populations with higher likelihood of *infection* can be preferentially sampled. Article 3.1. of the relevant disease-specific chapter of the *Aquatic Manual* should be used to inform sampling. There should be a gap of at least three months between surveys and, if there are breaks in production, the surveys should also ideally span two production cycles.

For a country or *zone* to regain freedom in accordance with pathway 4, the required period of *targeted surveillance* specified in the disease-specific chapter of the *Aquatic Code* will be consistent with the original self-declaration of freedom.

For *compartments*, the minimum default period that *targeted surveillance* should occur prior to a *self-declaration of freedom from disease* is one year. This shorter period for a *compartment* reflects the more clearly defined populations, the *biosecurity* required to maintain its population's health status and a likely narrower variation in environmental variables. However, a different period (more than one year) may be stipulated in the disease-specific chapter of the *Aquatic Code* if warranted by the epidemiology of the *disease* and the criteria proposed above. For example, different requirements may be appropriate where *susceptible species* have a three-year production cycle, versus one that has a six-month production cycle; particularly if the *disease* is likely to occur at a very low *prevalence* until near the end of the production cycle.

For *compartments* to regain freedom in accordance with pathway 4, the required period of *targeted surveillance* specified in the disease-specific chapter of the *Aquatic Code* may be less than the original declaration of freedom (dependent on the nature of the specific *disease* and as specified in the relevant disease-specific chapter). However, at least one survey in the *compartment* is required to demonstrate that eradication has been successful and to ensure the reviewed *basic biosecurity conditions* are effective.

Article 1.4.11.

Pathway 1 – Absence of susceptible species

Unless otherwise specified in the relevant disease-specific chapter of the *Aquatic Code*, a self-declaration of freedom from a specific *disease* may be made for a country or *zone* without applying *targeted surveillance* if there are no *susceptible species* (as listed in Article X.X.2. of the relevant disease-specific chapter of the *Aquatic Code*) present in that country or *zone*.

Basic biosecurity conditions should be in place for a period of time prior to a *self-declaration of freedom from disease*.

This pathway relies on confidence that *susceptible species* are in fact absent from a country or *zone*. To be confident that *susceptible species* are absent there should be:

1) sound knowledge of the range of *susceptible species* of a *pathogenic agent*; and

2) sufficient knowledge, of the local *aquatic animal* fauna (including wild populations) demonstrated by the following forms of evidence:

    a) reports which provide evidence regarding the absence of the *susceptible species* in the country or *zone* from structured surveys (e.g. of fisheries and aquatic fauna surveys, historical fisheries data);

    b) documentation from the relevant *Competent Authority* showing that those *susceptible species* have not been imported into the country or *zone*;

    c) provision of documentation which sets out scientific evidence indicating that the likelihood of the presence of *susceptible species* in the country or *zone* is negligible (e.g. data on physiological requirements, oceanographic information, biodiversity databases).

This pathway cannot be used for *diseases* where there is uncertainty regarding the full range of *susceptible species* (e.g. *diseases* with a broad host range), or where the *pathogenic agent* may not be obligate (e.g. able to survive indefinitely outside the host). In these cases, the pathway will be absent from the relevant disease-specific chapter of the *Aquatic Code*, and alternative pathways to demonstrate freedom should be utilised.

The pathway is intended primarily to be used by a *Competent Authority* wishing to establish freedom ahead of farming a new species.

Article 1.4.12.

Pathway 2 – Historical freedom

Unless otherwise specified in the relevant disease-specific chapter of the *Aquatic Code*, a *self-declaration of freedom from disease* may be made for a country or *zone* on the basis of historical freedom. The primary evidence for historical freedom is *passive surveillance* information generated by a country's *early detection system*. For this pathway to be utilised, the following conditions should be met:

1) the country or *zone* has *basic biosecurity conditions* in place, including an *early detection system*, that is sufficiently sensitive to detect the *disease* should it occur, and the requirements for *basic biosecurity conditions* of Article 1.4.6., *early detection system* of Article 1.4.7. and *passive surveillance* of Article 1.4.8. are met;

2) the *disease* has not been reported in the country or *zone* (including in wild *aquatic animal* populations) for the minimum period specified in the relevant disease-specific chapter of the *Aquatic Code*.

Requirements for passive surveillance

A *Competent Authority* making a *self-declaration of freedom from disease* on the basis of historical freedom will need to provide an explanation of how the criteria (i.e. for *basic biosecurity conditions*) presented for this pathway have been met. Specifically, a *Competent Authority* needs to provide evidence that its *early detection system* meets the conditions described in Article 1.4.7. and the requirements for *passive surveillance* in Article 1.4.8. The *early detection system* needs to represent all the *susceptible species* populations in the country or *zone*. If a *Competent Authority* cannot demonstrate that the required characteristics are fulfilled, due to a country's circumstances (e.g. nature of the *early detection system*, environmental conditions, nature of the *aquaculture*), this pathway is not considered valid. Instead, an alternative pathway that utilises *targeted surveillance* data will be required, or the *passive surveillance* information will need to be supplemented with *targeted surveillance* data (see below).

Need for targeted surveillance

If the requirements for *passive surveillance* specified in points 1 and 2 above would not be met for some defined populations of *susceptible species* (e.g. for wild populations), *targeted surveillance* may be used to provide additional evidence of freedom for those populations. Pathway 2 should only be utilised as the basis of a *self-declaration of freedom from disease*, if it is based primarily on *passive surveillance* information to demonstrate historical freedom; alternatively, pathway 3, as described in Article 1.4.13., should be used.

Article 1.4.13.

Pathway 3 – Targeted surveillance

As specified in the relevant disease-specific chapter of the *Aquatic Code*, a *self-declaration of freedom from disease* may be made for a country, a *zone* or a *compartment* where the primary evidence for freedom is *targeted surveillance* data. For this pathway to be utilised, the following conditions should be met:

1) prior to the commencement of *targeted surveillance*, *basic biosecurity conditions* have been in place for a default minimum period as specified in the relevant disease-specific chapter of the *Aquatic Code*;
2) the *disease* has not been reported in the country, *zone* or *compartment*, despite *targeted surveillance* that has been conducted for a period as specified in the relevant disease-specific chapter of the *Aquatic Code*, and in accordance with the requirements below.

Requirements for targeted surveillance

For many *diseases*, there will be significant temporal variability in the *prevalence* and intensity of *infection* (and therefore likelihood of detection by *targeted surveillance*). For example, the likelihood of detection may be greatest for a particular life stage, or during periods of the year when *pathogenic agent* replication and transmission are at their highest.

Environmental variability from one year to another may also result in differences in *prevalence* and intensity between years that could affect likelihood of detection. Surveys should therefore be designed to account for such variability and sample populations in a manner to maximise the likelihood of detecting a *disease* should it occur. This may require targeting temporal windows such that sampling can only take place during limited periods within a single year. Based on an assessment of potential pathways of introduction of the *diseases*, high risk regions or *aquaculture establishments* should be identified and preferentially included in the *surveillance* programmes. For example, establishments near ports or processing facilities may have higher likelihood of exposure to introduced *pathogenic agents*.

To maximise the likelihood of *pathogenic agent* detection, surveys should select species and life stages most likely to be infected and take place at times of the year when temperature and season offer the best opportunity for detection. At least two surveys per year (for at least two consecutive years – the default minimum period) need to be conducted three or more months apart to declare freedom unless disease-specific evidence supports an alternative strategy. In situations where seasonal conditions do not permit a gap of at least three months between surveys, the maximum possible time gap should be allowed to elapse between one survey and the next.

Over the period of *targeted surveillance*, the combined number of *aquaculture establishments* and *aquatic animals* sampled should be sufficient to generate at least 95% confidence that the *pathogenic agent* would be detected if present at or above the design *prevalence* in the country, *zone* or *compartment*. Design *prevalence* at the animal and higher levels of aggregation (i.e. pond, *aquaculture establishment*, village, etc.) should be set to a maximum of 2% (a higher design *prevalence* can only be used if justified by epidemiological evidence as described in Article 1.4.16.). Surveys should be designed in accordance with the recommendations provided in Article 1.4.16.

Other sources of data

This pathway to *disease* freedom should be based primarily on the results of *targeted surveillance*. However, the submission may also include an analysis of the *passive surveillance* information to provide supplemental evidence. This evidence may be used for defined populations of *susceptible species* where *passive surveillance* is demonstrated to be sufficiently sensitive (as described in Article 1.4.8.).

Article 1.4.14.

Pathway 4 – Returning to freedom

As specified in the relevant disease-specific chapter of the *Aquatic Code*, a *self-declaration of freedom from disease* may be made for a country, a *zone* or a *compartment* for which a self-declaration had previously been made, but subsequently lost due to an *outbreak* of the *disease*.

For a country or a *zone*, the default minimum period of *surveillance* to regain freedom is consistent with the requirements for pathway 3. However, a self-declaration of freedom can be made sooner if the relevant *Competent Authority* can demonstrate that the approach would provide an appropriate standard of evidence for the circumstances of the *outbreak* and the *disease*.

Compartments are able to return to freedom relatively rapidly; however, a minimum period of time is required as specified in each disease-specific chapter of the *Aquatic Code* to demonstrate that eradication has been successful and to ensure the reviewed *basic biosecurity conditions* are effective.

For a country, *zone* or *compartment*, a self-declaration utilising this pathway should provide information on the process employed to review and update *basic biosecurity conditions*. This information should also address the outcomes of the review and any relevant *sanitary measures* implemented to strengthen *basic biosecurity conditions*.

1. Infected zone and protection zone

Infected zones and *protection zones* should be established through exposure contact tracing from known infected *aquaculture establishments* (e.g. by following movements of *aquatic animals* or equipment to and from infected establishments) to identify all known infected establishments. Once contact tracing is complete and no new cases are being reported or detected through tracing, the boundaries of *infected zones* and *protection zones* can be finalised. The geographic extent of an *infected zone* should be based on the spatial distributions of infected and non-infected establishments within a region (e.g. river, estuary or bay). The *zone* should be defined to encompass geographically clustered infected populations.

The geographic extent of a *protection zone* needs to provide a very high level of confidence that measures implemented within the *zone* will prevent spread from the *zone* and should be based on the epidemiology of the transmissible *pathogenic agent*, the potential for exposure of neighbouring *aquaculture establishments*, the type of aquaculture production systems (e.g. open or closed systems), the influence of wild populations, and the local hydrology. In the marine environment, local hydrology (including tidal excursion), the distribution of suitable habitats for *susceptible species* and the movement of wild *susceptible species* or *vectors* should be considered. In the freshwater environment, the boundaries of the *protection zone* should be informed by the distance downstream that viable *pathogenic agent* is likely to spread on currents. If susceptible wild populations or *vectors* are present, their migratory patterns and ranges should be used.

Once *infected zones* and *protection zones* have been established, and no new cases have been detected for a period equal to or greater than the incubation period of the *pathogenic agent* (but no shorter than one month), the region outside of the *infected zones* and *protection zones* can be declared a *disease free zone*. Re-establishing *disease* freedom in the *infected zones* and *protection zones* requires *targeted surveillance*.

2. Requirements for targeted surveillance in a country or zone

Once all infected populations have been depopulated and affected *aquaculture establishments* have been disinfected, as described in Chapter 4.4., and synchronously fallowed as described in Chapter 4.7., for a period determined by the biophysical properties of the *pathogenic agent* (i.e. survival in the environment), a *surveillance* programme within the *protection zones* and *infected zones* should commence. The programme should include both farmed and wild populations of *susceptible species* in the *protection zones* and *infected zones*. A *risk*-based approach to the design of the survey is recommended (as described in Article 1.4.6.). The following *aquaculture establishments* or populations should be preferentially selected for sampling:

a) establishments which have been restocked following depopulation;

b) establishments and wild populations at greatest *risk* of exposure to *infection* during the *outbreak*, i.e. in close hydrographical proximity to infected establishments or with other epidemiological contacts such as sharing equipment or movements of *aquatic animals*;

c) wild populations of *susceptible species* downstream or in the immediate vicinity of previously infected establishments.

It is recommended that at least two negative surveys are conducted prior to reclaiming freedom. The second survey should start at least three months after completion of the first survey. Surveys should take place during optimum seasons, temperatures, and priority life stages to optimise *pathogenic agent* detection. If there are breaks in production, the surveys should also ideally span two production cycles. The number of *aquaculture establishments* and the samples taken per establishment in each survey should be sufficient to demonstrate with 95% confidence that the *pathogenic agent* would be detected if present above a *prevalence* of 2% (a higher design *prevalence* can be used if justified by epidemiological evidence). If *disease* is detected in wild populations of *susceptible species* and eradication is not possible, the country or *zone* remains infected.

3. Requirements for targeted surveillance in a compartment

Once the infected populations have been depopulated and affected *aquaculture establishments* disinfected, as described in Chapter 4.4. and fallowed as described in Chapter 4.7., for a period determined by the biophysical properties of the *pathogenic agent* (i.e. survival in the environment), the *compartment* can be restocked. A single survey is required following restocking to demonstrate that eradication has been successful. The survey should be undertaken at least sixth months, or at the maximum length of time allowed by the production cycle of species, after the *aquaculture establishment* has been restocked to ensure that the reviewed *basic biosecurity conditions* are effective. The survey should take place during optimum seasons, temperatures, and priority life stages to optimise *pathogenic agent* detection. The number of holding *units* (e.g. ponds, tanks) and the animals per holding *unit* sampled should be sufficient to demonstrate with 95% confidence that the *pathogenic agent* would be

detected above a *prevalence* of 2% (a higher design *prevalence* can be used if justified by epidemiological evidence).

Article 1.4.15.

Maintenance of disease free status

A country, *zone* or *compartment* that is declared free may maintain its free status provided that the *biosecurity* and *surveillance* requirements described in Article 1.4.5. are continuously maintained and the following requirements are met, as relevant:

1) For a country or *zone* with shared water bodies extending across the *territory* of other countries, free status can only be maintained if the requirements to maintain freedom are in place across all epidemiologically linked shared water bodies.
2) A country, *zone* or *compartment* declared free may maintain its free status without *targeted surveillance* provided that the requirements for *passive surveillance* in Article 1.4.8. are met for the entire country, *zone* or *compartment*, and in the case of:
   a) a declared free *zone*, the *zone* occurs within the *territory* of a country declared free;
   b) a declared free *compartment*, the *compartment* occurs within the *territory* of a country declared free.
3) If the conditions of point 2 are not met, ongoing *targeted surveillance* for the *pathogenic agent*, as described in Article 1.4.16., is required at a level determined by a *Competent Authority*, to generate an annual 95% confidence of detection, taking into account the likelihood of *infection*.
4) *Competent Authorities* should ensure prompt investigation of any health events or other information that may raise suspicion of the occurrence of a *listed disease* from which a country, *zone* or *compartment* has been declared free. The investigation should be undertaken in accordance with Article 1.4.18. and the requirements of Chapters 1.1. and 5.1. should be met at all times.

Article 1.4.16.

Design of surveys to demonstrate freedom from disease

Surveys to demonstrate freedom from a specified *disease* (i.e. *targeted surveillance*) are required for pathway 3 as described in Article 1.4.13. to achieve a *disease* free status, and to regain a *disease* free status following detection of the *pathogenic agent* as described in Article 1.4.14. and to maintain *disease* freedom. Surveys may be required to supplement *passive surveillance* information generated by the *early detection system* required for pathway 2 as described in Article 1.4.12. In addition, where conditions are not conducive to clinical expression of *disease*, and, therefore, the *early detection system* cannot provide evidence for the maintenance of freedom, ongoing *targeted surveillance* is required.

It is not possible to provide absolute certainty of the absence of *disease*. Surveys can demonstrate freedom from *disease* by generating evidence that a *disease* is not present in a population at or above a predetermined *prevalence* (the design *prevalence*) and to an acceptable level of confidence. Apparent *disease* at any level in the *target population* automatically invalidates any freedom from *disease* claim, unless, on the basis of further testing, positive test results are accepted as false positives. A survey to demonstrate freedom from *disease* should meet the following requirements set out in this article:

1. Population

   The population of *epidemiological units* should be clearly defined. *Aquaculture establishments* and holding *units* (e.g. ponds, tanks) within establishments are the most commonly used *epidemiological unit* in surveys to demonstrate *disease* freedom. It is, therefore, important that *Competent Authorities* should keep registries of *aquaculture establishments*, which include geographic location and species held.

   The *target population* consists of all individuals within the selected population of *susceptible species* to the *disease* in a country, *zone* or *compartment*, to which the *surveillance* results apply. *Disease* introduction may be more likely to occur in some components of the *target population* than others. In these cases, it is advisable to focus *surveillance* efforts on this part of the population.

   The design of the survey will depend on the size and structure of the population being studied. If the population can be considered to be homogenous with regards to likelihood of exposure, a single-stage survey can be used.

   Farmed *aquatic animals* are not individually identified and usually kept in holding *units* (e.g. ponds, tanks) which can lead to clusters of *infection* within *aquaculture establishments*. Similarly, wild *aquatic animal* populations are not evenly distributed within a *zone*. For these reasons, multi-stage sampling is recommended. In two-stage

sampling, at the first stage of sampling, groups of animals (e.g. *aquaculture establishments* or villages) are selected. At the second stage, animals are selected for testing from each of the first-stage sampling groups.

In the case of a complex (e.g. multi-level) population structure, multi-stage sampling may be used, and the data analysed accordingly.

2. Dossier of evidence

The sources of evidence should be fully described. A survey should include a description of the sampling strategy used for the selection of *units* for testing. For complex *surveillance* systems, a full description of the system is required, including consideration of any *biases* that may be inherent in the system. Evidence to support claims of freedom from *disease* can use non-random sources of information, provided that, overall, any *biases* introduced subsequently favour the detection.

3. Statistical methodology

The analysis and interpretation of test results from a survey shall be in accordance with the provisions of this chapter and consider the following factors:

a) the survey design;

b) the diagnostic *sensitivity* and *specificity* of the test or test system;

c) the design *prevalence* (or *prevalences* where a multi-stage design is used).

Analysis of data for evidence of freedom from *disease* involves estimating the probability (alpha) that the evidence observed (i.e. negative results for *disease* detection from *surveillance*) could have been produced assuming that *infection* is present in the population at or above the minimum specified *prevalence* (the design *prevalence*). The confidence in (or, equivalently, the *sensitivity* of) the survey that produced the evidence is equal to 1-alpha. If the confidence level exceeds a pre-set threshold, the evidence is deemed adequate to demonstrate freedom from *infection*. The required level of confidence (that the survey would detect *infection* if *infection* were present at or above the specified level) should be equal to or greater than 95%.

The power (probability that the survey would report that no *infection* is present if *infection* is truly not present) is by convention set to 80%, but may be adjusted in accordance with the country's or *zone*'s requirements.

Statistical analysis of *surveillance* data often requires assumptions about population parameters or test characteristics. These are usually based on expert opinion, previous studies on the same or similar populations, and epidemiology of the *disease*.

The values for design *prevalence* used in calculations should be based on the epidemiology of the *disease*. Justification for the selection of design *prevalence* values should be provided, and should be based on the following recommendations:

a) At the individual animal level (e.g. *prevalence* of infected animals in a pond, tank or net pen, or cages), the design *prevalence* is based on the epidemiology of the *infection* in the population. It is equal to the minimum expected *prevalence* of *infection* in the *study population*, if the *infection* had become established in that population. A suitable design *prevalence* value at the animal level may be:

   i) between 1% and 5% for *infections* that are present in a small part of the population, e.g. are transmitted slowly or have been recently introduced, etc.;

   ii) over 5% for highly transmissible and persistent *infections*;

   iii) if reliable information, including expert opinion, on the expected *prevalence* in an infected population is not available, a value of 2% should be used for the design *prevalence*.

b) At higher levels (e.g. net pen or cage, pond, *aquaculture establishments*, village, etc.) the design *prevalence* should be based on empirical evidence and reflect the expected behaviour of the *infection*. A higher establishment-level design *prevalence* can be used for *diseases* which spread rapidly between pens or cages, and establishments. *Diseases* which are transient or less contagious require lower design *prevalences*:

   i) a suitable design *prevalence* value for the first level of clustering (e.g. proportion of infected establishments in a *zone*) is normally not greater than 2%. If a higher design *prevalence* is selected, it should be justified.

4. Risk-based sampling

Risk-based sampling is an approach to identify and sample populations that have the greatest likelihood of *infection*. It can be applied to the design of surveys to demonstrate freedom from *disease* for a country, *zone* or *compartment*. A key advantage of *risk*-based sampling is that it can improve the efficiency of *surveillance* to demonstrate freedom from *disease* compared to random sampling approaches.

Risk-based sampling requires the identification of *risk* factors that are applied to *bias* sample collection to populations of *aquatic animals* considered most likely to be infected if the specific *disease* had been introduced and had established. Where *risk*-based sampling is used for demonstration of freedom, the *risk* factors that

underpin survey design, and the evidence or assumptions for their selection, should be documented. Where existing *risk assessments* are available, these may be utilised to identify *risk* factors associated with *disease* introduction, exposure and establishment. The identification of appropriate *risk* factors may include consideration of:

a) the possible pathways of *disease* introduction (e.g. through *aquatic animals*, *aquatic animal products*, *feed*, fomites, *vectors* and water);

b) proximity of susceptible populations to sources of *disease* exposure (e.g. to *aquatic animal* processing facilities, or ports);

c) environmental or husbandry conditions that are permissive for *disease* establishment (e.g. temperature, salinity, production system type, habitat type, exposure to recent stressors);

d) conditions that are conducive for development of clinical *disease*; including the species or life stages that are most susceptible to clinical *disease*;

e) evidence of morbidity or mortality.

5. Test characteristics

All *surveillance* involves performing one or more tests for evidence of the presence of current or past *infection*, ranging from laboratory assays to farmer observations. The performance level of a test is described in terms of its diagnostic *sensitivity* and *specificity*. Imperfect *sensitivity* or *specificity* impact on the interpretation of *surveillance* results, and should be taken into account in the analysis of *surveillance* data. For example, in the case of a test with imperfect diagnostic *specificity*, if the population is free of *disease* or has a very low *prevalence* of *infection*, all or a large proportion of positive tests will be false. Samples that test positive should be confirmed or refuted using a second highly specific test. Where more than one test is used (sometimes called using tests in series or parallel), the *sensitivity* and *specificity* of the test combination should be calculated.

All calculations should take the performance level (*sensitivity* and *specificity*) of any tests used into account. Information on test characteristics provided in the relevant disease-specific chapter of the *Aquatic Manual* should be used unless more appropriate information is available. The estimate of test *sensitivity* when the test was used in apparently healthy *aquatic animals* should be used. Samples should not be pooled before testing, unless approved in the relevant disease-specific chapter of the *Aquatic Manual*. If pooled testing is used, the results of testing should be interpreted using *sensitivity* and *specificity* values that have been determined or estimated for that particular pooled testing procedure, and for the applicable pool sizes being used.

6. Sample size

In surveys conducted to demonstrate the absence or presence of an *infection*, the number of units to be sampled from a population should be calculated, using a statistically valid technique that takes at least the following factors into account:

a) the *sensitivity* and *specificity* of the diagnostic test,

b) the design *prevalence* (or *prevalences* where a multi-stage design is used),

c) the level of confidence that is desired of the survey results.

Additionally, other factors may be considered in sample size calculations, including (but not limited to):

a) the size of the population (but it is acceptable to assume that the population is infinitely large),

b) the desired power of the survey.

Software for the calculation of sample sizes at varying parameter values are available. Table 1.2. provides examples of sample sizes generated by the software for a type I and type II error of 5% (i.e. 95% confidence and 95% statistical power). However, this does not mean that a type 1 and type 2 error of 0.05 should always be used. For example, using a test with *sensitivity* and *specificity* of 99%, 528 *units* should be sampled. If nine or less of those *units* test positive, the population can still be considered free of the *infection* at a design *prevalence* of 2%, provided that all efforts are made to ensure that all presumed false positives are indeed false (i.e. by use of a second highly specific assay). This means that there is a 95% confidence that the *prevalence* is 2% or lower, which reflects the fact that false negative results can occur. Incorrectly concluding that a population is free can be reduced by increasing the sample size and using more than one assay but cannot be completely eliminated.

In the case in which the values of *sensitivity* and *specificity* are not known (e.g. no information is available in the relevant disease-specific chapter of the *Aquatic Manual*), they should not automatically be assumed to be 100%. All positive results should be included and discussed in any report regarding that particular survey, and all efforts should be made to ensure that all presumed false positives are indeed false.

7. Multi-stage structured survey design

In general, a survey to demonstrate freedom at *zone* or country level should use a multi-stage design. The first sampling level is often *aquaculture establishments* (or villages) or populations of wild *susceptible species*, and the

second stage may be ponds or individual animals within the establishment (or village) or defined stocks within a wild population. At each level, design levels need to be set and sample sizes calculated.

8. Quality assurance

Surveys should include a documented quality assurance system, to ensure that field and other procedures conform to the specified survey design. Acceptable systems may be quite simple, as long as they provide verifiable documentation of procedures and basic checks to detect significant deviations of procedures from those documented in the survey design.

Table 1.2. Sample sizes for different design *prevalences* and test characteristics.

Design prevalence (%)	Sensitivity (%)	Specificity (%)	Sample size	Maximum number of false positive if the population is free
2	100	100	149	0
2	100	99	524	9
2	100	95	1 671	98
2	99	100	150	0
2	99	99	528	9
2	99	95	1 707	100
2	95	100	157	0
2	95	99	542	9
2	95	95	1 854	108
2	90	100	165	0
2	90	99	607	10
2	90	95	2 059	119
2	80	100	186	0
2	80	99	750	12
2	80	95	2 599	148
5	100	100	59	0
5	100	99	128	3
5	100	95	330	23
5	99	100	59	0
5	99	99	129	3
5	99	95	331	23
5	95	100	62	0
5	95	99	134	3
5	95	95	351	24
5	90	100	66	0
5	90	99	166	4
5	90	95	398	27
5	80	100	74	0
5	80	99	183	4
5	80	95	486	32

Article 1.4.17.

Combining multiple sources of information

Pathway 1 to achieving *disease* freedom (absence of *susceptible species*) relies on a range of data sources. Pathway 2 to achieving *disease* freedom (historical freedom) will primarily use evidence from *passive surveillance*, which may come from multiple sources (as described in Article 1.4.8.) and may be supplemented with *targeted surveillance* if necessary (as described in Article 1.4.12.). *Passive surveillance* information can also be used to provide additional support for *disease* freedom, based on *targeted surveillance* (i.e. pathway 3). Estimates of the confidence in each data source may be combined to provide an overall level of confidence of freedom from *disease* for the combined data sources. The methodology used to combine the estimates from multiple data sources:

1) should be scientifically valid and fully documented, including references to published material; and

2) should, where possible, take into account any lack of statistical independence between different data sources.

If combining evidence from different sources including *passive surveillance* and *targeted surveillance*, a *Competent Authority* may choose to use various approaches, such as a scenario tree modelling approach.

Article 1.4.18.

Diagnostic confirmation of a listed disease or an emerging disease

A *Competent Authority* is required to provide *disease notifications* as described in Chapter 1.1.

The relevant disease-specific chapter of the *Aquatic Manual* provides recommendations for the appropriate diagnostic methods for presumptive and confirmatory diagnostic purposes. The assays recommended for these purposes are presented in Table 4.1 of the relevant disease-specific chapter of the *Aquatic Manual*.

The recommended standards of diagnostic evidence to confirm *infection* in either apparently healthy or clinically diseased animals are provided in Section 6 of the relevant disease-specific chapter of the *Aquatic Manual*. These case definitions for suspect and confirmed cases have been developed to support decision making in relation to trade and for confirmation of *disease* status at the level of a country, *zone* or *compartment*. A *Competent Authority* may choose to apply a lower standard of evidence for *disease* confirmation within its *territory* for known endemic *diseases*.

If standards of evidence are not met to confirm a suspect case of *disease* in accordance with the case definitions in Section 6 of the relevant disease-specific chapter of the *Aquatic Manual*, ongoing investigation is required until sufficient evidence is obtained to either:

1) exclude the presence of a *listed disease* or an *emerging disease*; or

2) to confirm the presence of a *listed disease* or an *emerging disease*.

If a Member Country does not have access to a laboratory with the capability to undertake the necessary diagnostic tests and which meets the requirements of Chapter 1.1.1. of the *Aquatic Manual* it should seek advice from the relevant WOAH Reference Laboratory.

In all circumstances, Member Countries should comply with the requirements described in Chapter 1.1. to provide transparent and timely *notifications* to allow Member Countries to take appropriate action to prevent the transboundary spread of important *diseases* of *aquatic animals*.

NB: FIRST ADOPTED IN 2008; MOST RECENT UPDATE ADOPTED IN 2022.