EURL
FOR FISH
DISEASES

SURVEILLANCE AND CONTROL METHODS FOR INFECTIOUS SALMON ANEMIA (ISA)

PART 3

SURVEILLANCE AND CONTROL METHODS FOR INFECTIOUS SALMON ANEMIA (ISA)

I. **Requirements for surveillance and eradication programmes to obtain and to maintain disease-free health statuses with regard to ISA and to contain infection with HPR deleted ISAV**

I.1. General requirements

When health inspections and sampling of farms in accordance with the second paragraph of point 2 of part I of Annex V to Directive 2006/88/EC is required to be carried out more than once per year, the intervals between the health inspections or collection of samples shall be as long as possible.

When targeted surveillance in wild populations is required in accordance with the second paragraph of point 2 of Part I of Annex V to Directive 2006/88/EC, the number and geographical distribution of sampling points shall be determined to obtain a reasonable coverage of the Member State, zone or compartment. The sampling points shall also be representative of the different ecosystems where the wild susceptible populations are located.

The health inspections shall be carried out in all production units, such as ponds, tanks and net cages, for the presence of dead, weak or abnormally behaving fish. Particular attention shall be paid to the water outlet area where weak fish tend to accumulate because of the water current.

The fish to be collected as samples shall be selected as follows:

(a) only moribund or freshly dead fish, but not decomposed fish shall be selected; in particular fish demonstrating anaemia, bleedings or other clinical signs suggesting circulatory disturbances shall be prioritised for collection;

(b) if Atlantic salmon is among the susceptible species on the site, samples from Atlantic salmon shall be prioritised. If there is no Atlantic salmon in the fish farm, other susceptible species must be sampled;

(c) if more than one water source is utilised for fish production, fish representing all water sources shall be included in the sample;

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

I.2. Specific requirements to achieve Category I health status with regard to ISA

I.2.1. Surveillance programmes

A Member State, zone or compartment which has Category III health status in accordance with part B of Annex III to Directive 2006/88/EC with regard to ISA may achieve Category I health status with regard to that listed disease when all farms keeping susceptible species listed in Part II of Annex IV to Directive 2006/88/EC within the Member State, zone or compartment meet the relevant requirements set out in Annex V to that Directive and all those farms and, when required by the second paragraph of point 2 of Part I of Annex V thereto, sampling points in wild populations selected in accordance with that point, have been subject to the following surveillance programme:

(a) the farms or sampling points have been subject to health inspections and sampled for a minimum period of two consecutive years as laid down in Table 3.A set out in Section II;

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

(c) if during the implementation of the surveillance programme, ISA is confirmed in a farm included in that surveillance programme, and therefore its Category II health status has been withdrawn, an eradication programme in accordance with point I.2.2 must have been carried out.

I.2.2. Eradication programmes

I.2.2.1. General requirements

A Member State, zone or a compartment that has Category V health status with regard to ISA may achieve Category I health status with regard to that listed disease when all farms keeping susceptible species listed in Part II of Annex IV to Directive 2006/88/EC within the Member State, zone or compartment have been subject to an eradication programme that complies with the following points (a) to (e).

(a) the minimum control measures laid down in Section 3 of Chapter V of Directive 2006/88/EC have effectively been applied, and in particular a containment area as referred to in Article 32(b) of that Directive, comprising a protection zone and surveillance zone, has been established in the vicinity of the farm(s) officially declared infected with HPR-deleted ISAV or confirmed ISA.

The containment area must have been defined on a case-by-case basis taking into account factors influencing the risks for the spread of ISA to farmed or wild fish, such as: the number, rate and distribution of the mortality of fish on the farm infected with HPR-deleted ISAV or confirmed ISA; the distance and density of neighbouring farms; the proximity to slaughterhouses; contact farms; the species present at the farms; the farming practices applied in the affected and neighbouring farms; the hydrodynamic conditions and other factors of epidemiological significance identified.

For the establishment of the protection and surveillance zones, the following minimum requirements shall apply as regards the geographical demarcation of those zones:

(i) a protection zone shall be established in the immediate vicinity of a farm officially declared infected with ISA and shall correspond to:

(1) in coastal areas: 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 farm officially declared infected with ISA, or an equivalent area determined according to appropriate hydrodynamic or epidemiological data;

(2) in inland areas: the entire water catchment area of the farm officially declared infected with ISA; the competent authority may limit the extension of the zone to parts of the water catchment area provided that the prevention of the spread ISA is not compromised;

(ii) a surveillance zone shall be established outside the protection zone and shall correspond to:

(1) in coastal areas: 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 10 km from the centre of the protection zone; or an equivalent area determined according to appropriate hydrodynamic or epidemiological data; or

(2) in inland areas: an extended area outside the established protection zone;

(b) all farms keeping susceptible species listed in Part II of Annex IV to Directive 2006/88/EC within the protection zone not officially declared infected with ISA shall be subject to an official 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 signs consistent with ISA are observed, or minimum 30 fish when no clinical or post mortem signs are observed;

(ii) one health inspection; in those farms where the tests referred to in (i) have produced negative results, the health inspections shall continue once per month until the protection zone is withdrawn in accordance with point I.2.2.1(c);

(c) all farms officially declared infected with HPR-deleted ISAV or confirmed ISA shall be emptied, cleansed, disinfected and fallowed for a period of at least 3 months. The protection and surveillance zones may be lifted when all farms within the protection zone are emptied, cleaned, disinfected and followed by a synchronized fallowing period of at least 6 weeks.

When fallowing of the officially declared infected farms is carried out, the protection zones shall be converted into surveillance zones.

The competent authority may decide to require the emptying, cleansing, disinfection and fallowing of other farms within the established protection and surveillance zones. The length of the fallowing period for those farms shall be determined by the competent authority following a case-by-case risk evaluation;

(d) all farms officially declared infected with HPR-deleted ISAV or confirmed ISA and all other farms fallowed within the established protection and surveillance zones shall be restocked with fish sourced from Member States, zones or compartments with a Category I with regard to ISA.

Restocking shall only take place when all farms officially declared infected have been emptied, cleansed, disinfected and fallowed in accordance with point I.2.2.1(c);

(e) all farms keeping susceptible species listed in Part II of Annex IV to Directive 2006/88/EC within the member State, zone or compartment covered by the eradication programme and when surveillance in wild populations is required, sampling points selected in accordance with point I.1, shall subsequently be subject to the surveillance scheme set out in point I.2.1.

I.2.2.2. Requirements concerning regaining disease-free status for continental compartments comprising one single farm that was previously declared as having Category I health status

A continental compartment comprising one single farm that has Category I health status with regard to ISA, whose health status is independent of the surrounding natural waters in accordance with point 3 of Part II of Annex V to Directive 2006/88/EC, and whose Category I health status has been withdrawn in accordance with Article 53(3) of that Directive, may regain it again immediately after the competent authority has confirmed that it complies with the following conditions:

(a) it has been emptied, cleansed, disinfected and fallowed; the duration of the fallowing period shall be at least 6 weeks;

(b) it has been restocked with fish sourced from Member States, zones or compartments with a Category I health status as regards ISA.

I.3. Minimum control measures for the maintenance of Category I status with regard to ISA

When targeted surveillance is required to maintain Category I health status, as provided for in Article 52 of Directive 2006/88/EC, all farms keeping susceptible species listed in Part II of Annex IV to that Directive within the Member State, zone or compartment concerned shall be subject to health inspections and sampled in accordance with Table 3.B [1] set out in Section II of this Part, taking into account the risk level of the farm for the contraction of ISA.

When determining the health inspection frequency for category I health status with regard to ISA for compartments which are placed in continental areas and where the health status with regard to ISA is dependent of the health status of surrounding natural waters that are housing Atlantic salmon (*Salmo salar*), the risk for the contraction of ISA shall be regarded as high.

Disease-free status with regard to ISA may only be maintained as long as all samples tested using the diagnostic methods set out in point II.2 have produced negative results for HPR-deleted ISAV and any suspicion of ISA has been ruled out in accordance with the diagnostic methods set out in point II.3.

I.4. Specific requirements to achieve Category III health status with regard to HPR-deleted ISAV in Member States, zones or compartments that previously held Category V health status

A Member State, zone or compartment that has Category V health status with regard to ISA may achieve Category III status provided that:

(a) the requirements set out in point I.2.2.1 (a), (b) and (c) have been met. In case fallowing is not technically possible, the farms shall be subject to an alternative measure which provides almost similar guarantee for extermination of ISAV from the environment of the farm;

(b) all farms officially declared infected and all other farms fallowed or been subject to alternative measures in accordance with (a) within the protection and surveillance zones established, have been restocked with fish sourced from Member States, zones or compartments with a Category I, II or III health status with regard to ISA;

(c) that such restocking has only taken place after all farms officially declared infected have been emptied, cleansed, disinfected and fallowed/been subject to alternative measures in accordance with (a).

(d) no confirmation of HPR-deleted ISAV has occurred during the period of 2 years that follows the completion of the measures referred to in (a), (b) and (c), and suspicions during this period have been ruled out in accordance with the procedures established in point II.3.

[1] Shall not apply to farms only rearing rainbow trout (*Oncorhynchus mykiss*) or brown trout (*Salmo trutta*) or both and where the water supply is exclusively based on fresh water sources not housing Atlantic salmon (*Salmo salar*).

II. **Diagnostic methods and official investigations**

II.1. Samples

The tissue material to be examined shall be:

(a) Histology: head-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.

II.2. Diagnostic methods to obtain or maintain disease-free status with regard to ISA

The diagnostic method to be used to obtain or to maintain disease-free status with regard to ISA in accordance with points I.2 and I.3 shall be RT-qPCR, followed by sequencing of positive samples in accordance with the detailed methods and procedures set out in Part 3 of Annex II.

In the case of a positive result to RT-qPCR, further samples shall be tested before the implementation of the initial control measures provided for in Article 28 of Directive 2006/88/EC.

Those samples shall be tested as follows in accordance with the detailed methods and procedures set out in Part 3 of Annex II:

(a) screening of the samples by RT-qPCR, including sequencing of the HE-gene to verify HPR-deletion;

and

(b) examination in tissue preparations by means of specific antibodies against ISAV (namely IHC on fixed sections or IFAT on tissue imprints); or

(c) isolation and identification of ISAV in cell culture from at least one sample from any fish sampled from the farm.

II.3. Official investigation and diagnostic methods to rule out or to confirm the presence of ISA

When a suspicion of ISA shall be confirmed or ruled out in accordance with Article 28 of Directive 2006/88/EC, the following inspection, sampling and testing procedure shall be complied with:

(a) the official investigation which shall include at least one health inspection and one sampling of 10 moribund fish, when clinical signs or *post-mortem* signs consistent with ISA are observed. If no clinical signs or *post-mortem* signs consistent with ISA are observed, the health inspection shall be followed by targeted sampling of minimum 30 moribund fish or fresh *post-mortems* with normal constitution in accordance with point I.1. Samples shall be tested in accordance with the diagnostic methods set out in point (b);

(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 28 of Directive 2006/88/EC. A suspected case of infection with ISA shall be confirmed in accordance with the following criteria using the detailed methods and procedures set out in Part 3 of Annex II:

(i) Detection of ISAV by RT-qPCR, including sequencing of the HE-gene to verify HPR-deletion, and detection of ISAV in tissue preparations by means of specific antibodies against ISAV (namely IHC on fixed sections or IFAT on tissue imprints)

or

(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 farm;

(c) where the presence of clinical, gross pathological changes or histopathological findings consistent with ISA 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 Part 3 of Annex II.

The suspicion of ISA may be ruled out, if tests and inspections over a period of 12 months from the date of the suspicion are found to reveal no further evidence of the presence of ISA.

Table 3.A

Surveillance scheme for zones and compartments for the 2-year control period which precedes the achievement of disease-free status for ISA as referred to in point I.2.1

Year of surveillance	Number of health inspections per year (2 years)	Number of laboratory examinations per year (2 years)	Number of fish to be sampled per year
Year 1	6	2 (1)	2 * 75 (2)
Year 2	6	2 (1)	2 * 75 (2)

(1) Samples must be collected and stored and examined during two 1 month test periods per year (namely spring and autumn) or when appropriate in accordance with practical considerations.
(2) Maximum number of fish per pool: 5.

Table 3.B

Surveillance schemes for zones or compartments to maintain disease- free status for ISA as referred to in point I.3 (2)

Risk level	Number of health inspections per year	Number of laboratory examinations per year	Number of fish to be sampled per year
High	2	2 (1)	2 * 30
Medium	1	1 (1)	30
Low	1 every 2 years	1 every 2 years	30 every 2 years

(1) Samples must be collected and examined during two 1 month test periods per year (namely spring and autumn) or when appropriate in accordance with practical considerations.
(2) Shall not apply to farms only rearing rainbow trout (*Oncorhynchus mykiss*) or brown trout (*Salmo trutta*) or both and where the water supply is exclusively based on fresh water sources not housing Atlantic salmon (*Salmo salar*).