ISA HSP

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Infectious Salmon Anaemia (ISA)/
Infectious Salmon Anaemia Virus (ISAV)
Hazard Specific Plan

Draft Version 1.0

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Table of Contents

1.0 About this Document

1.1 Goals and Objectives

Infectious Salmon Anaemia is a Reportable aquatic animal disease in Canada. ISAV does occur in Canada; the current known geographical range is the susceptible aquatic animals and waters of New Brunswick and Prince Edward Island.

Disease response will vary depending on whether the strain of ISAV can be cultured or not.

- No disease response is planned for non-culturable strains of ISAV in enzootic areas or premises with enzootic ISAV. Currently the known avirulent strain is HPR0/North American lineage which has been found in cultured Atlantic salmon in New Brunswick.

The goals of Infectious Salmon Anaemia (ISA)/ Infectious Salmon Anaemia Virus (ISAV) Hazard Specific Plan (ISAV HSP) are to eradicate incursions of ISAV, or if eradication is not possible, to prevent the further spread of ISAV.

- For an incursion in populations of cultured finfish the primary disease control goal is restoration of ISAV-disease freedom[1]

- If freedom is not achievable in populations of cultured finfish, the goal is to control high risk movements of finfish susceptible to ISAV[2]

- For incursions in populations of wild finfish, the primary disease control goal is control of high risk movements of harvested wild finfish infected with or susceptible to ISAV

- In certain exceptional circumstances involving wild finfish, such as in closed watersheds, the primary disease control goal may be ISAV-disease freedom[3].

In order to meet the goals, the objectives of the ISAV HSP are to:

- provide specific infectious disease control policy statements, information and procedures that will eradicate ISAV from populations of cultured finfish where freedom is achievable.

- provide specific infectious disease control policy statements, information and procedures that will prevent the spread of ISAV within and between populations of finfish. Consideration must be given to animals, water, feed, things (fomites) and vectors as sources of ISAV.

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[1] In government hatcheries or aquaculture sites where the cultured finfish are ultimately released into the natural environment (thereby becoming wild finfish populations), decisions regarding disease response activities, such as depopulation, will be made in partnership with the government that has jurisdiction over that natural resource.
[2] Movements will be controlled either through the NAAHP Domestic Movement Permit Program or through the issuance of licences.
[3] Decisions involving disease response in wild finfish populations are made between CFIA and the jurisdiction that has responsibility for the natural resource (see Chapter 6 of the Aquatic Animal Health Functional Plan (AAHFP)

More specifically, ISAV disease control and response measures need to:

- identify all exposed populations of susceptible species of finfish, AND/OR
- prevent contact between susceptible populations of finfish and ISAV, AND/OR
- stop the spread or transmission (replication and/or release) of ISAV by infected finfish by:
  - stopping high risk movements of known and suspect infected populations of finfish
  - tracking all movements, in and out, of all life stages of susceptible species of finfish, including germplasm, carcasses , water, things, aquatic animal feed, and vectors within the 6 months prior to detection of the incursion. Priority is given to movements of aquatic animals, including germplasm, carcasses (including parts thereof), and offal, and those aquatic animals used for live or raw, potentially minced, aquatic animal feed, and movements of water (including the water associated with transports of aquatic animals).
  - inspection and testing of populations of finfish that have had direct exposure (primarily through aquatic animals or effluent water) to ISAV
  - destruction and disposal of known infected populations of finfish and disposing of the carcasses in a manner that prevents spread of ISAV to non-exposed populations of finfish, things (including water), aquatic animal feed, fomites and vectors
  - inactivation of ISAV by cleaning and/or disinfection of finfish eggs and fomites (including water)
  - inactivation of ISAV in aquatic animal carcasses (and parts thereof), germplasm, and offal
  - eliminating ISAV by destruction and/or disposal of contaminated things (including water) that cannot be cleaned and disinfected in a manner that prevents spread of ISAV
  - preventing vector access to: susceptible species of finfish, water used for susceptible species of finfish, aquatic animal feed, and fomites.

1.2 Authority

Reportable Diseases Regulations (enabled by the *Health of Animals Act*)
http://laws.justice.gc.ca/en/H-3.3/SOR-91-2
ISAV is a Reportable aquatic animal disease.

Please see Aquatic Animal Health Functional Plan, Chapter 1 for authorities for disease control and response.

1.3 Applicability

The ISAV HSP is for the use of staff in CFIA Programs and Operations when a notification of an ISAV incursion has been made to CFIA and a disease response is indicated.

1.4 Related Documents

The ISAV HSP is to be used in conjunction with the Aquatic Animal Health Functional Plan (AAHFP). The ISAV HSP provides policies, information and procedures specific to controlling ISAV, whereas non-disease specific policies and procedures related to aquatic animal disease control are contained in the AAHFP.

Name of Document	Merlin Link	RDIMS #
Aquatic Animal Health Functional Plan		2532911
ISAV Hazard Characterization	Under development	
ISAV Technical Disease Factsheet		2682000
Call Log/AquaPIQ	eForms Catalogue	
Suspect and Inspection Phases of Disease Response – Collection of Information		2719883
Inspection Phase/Action Phase of Disease Response: Sampling Cultured Finfish		2230752
NAAHP Laboratory Submission Form		2032376
How to Fill in the NAAHP Laboratory Submission Form		

1.5 Amendments and Revisions

It is the responsibility of Disease Control Contingency Planning (DCCP) section of the Aquatic Animal Health Division (AAHD) of CFIA to amend and revise the ISAV HSP.

The ISAV HSP will be reviewed and amended or revised, as necessary.

The ISAV HSP will be reviewed periodically by stakeholders and partners through the Aquatic Animal Health Committee.

Policy statements that address ISAV disease control responses in the various delineated zones will be reviewed by CFIA, stakeholders and partners each time a map of the ISAV health status of an Eradication Area is in the consultation process.

Suggestions and recommendations should be forwarded to:

National Manager, Disease Control Contingency Planning
Aquatic Animal Health Division
Canadian Food Inspection Agency
59 Camelot Drive
Ottawa, ON, K1A 0Y9
Email: NAAHPPNSAA@inspection.gc.ca
Tel: 613-773-7428
Fax: 613-773-7567

1.6 Document Distribution

For CFIA staff, this document is available on Merlin.

This document or a summary of this document will be available to Canadians on the CFIA web site.

1.7 ISA/ISAV Case Definitions

In finfish populations with clinical signs, the tests run by NAAHLS will be virus isolation and identification of the virus by a PCR method. In finfish populations that are apparently healthy, the tests run by NAAHLS will be screening with a PCR method. All positive tests will be confirmed by either virus isolation or another PCR method that targets a different segment of the viral genome.

Therefore, in order to detect all strains of virulent ISAV, specimens to be tested using virus isolation include heart and anterior kidney. Spleen and ovarian fluid may also be added to the list of specimens to test.

For detection of non-culturable strains (avirulent) of ISAV in apparently healthy populations of finfish, gill tissue must also be sampled; in addition, anterior kidney and heart tissue specimens will be collected to detect virulent strains of ISAV (ovarian fluid may also be collected to detect virulent strains of ISAV).

1.7.1 For the purpose of determining a High Probability of Infection with ISAV (HIGH RISK)

NOTE: Information required to make the determination of probability of infection may be recorded in AquaPIQ Part A and/or AquaPIQ Part B.

NOTE: Only test results from a NAAHLS laboratory are confirmatory.

Suspected ISAV detection in finfish with no clinical signs (individual fish or pooled samples)

An ISAV detection in a population of finfish will be suspected, and therefore the premises is considered HIGH RISK, when laboratory results include one or more of the following:

1. Isolation in cell culture and identification of ISAV by a non-CFIA approved laboratory[4].
2. Detection of ISAV antigen using one or more tests other than cell culture by a non-CFIA approved laboratory[4].
3. Detection of antibodies to ISAV by a non-CFIA approved laboratory.

[4] For reporting purposes to the OIE, the detection will be considered confirmed by CFIA if the detection occurs in a population where ISAV is considered enzootic (where disease response is not planned) and the laboratory has a veterinarian on staff. This does not apply to a detection in a new species.

Suspected ISA outbreak in finfish (individual fish or pooled samples)

Peracute disease has not been described in field cases of ISA. The mortality pattern is not pathognomonic. The pattern can either be acute with a rapid epidemic curve or chronic with low grade mortality (however, above expected values).

There are no pathognomonic signs for ISA, however, anemia and circulatory disturbances are always present during an outbreak.

An ISA outbreak in a population of finfish will be suspected, and therefore the premises is considered HIGH RISK, when a noticeable portion of the population (at least 10%) exhibits two or more of the following (clinical signs and one or more pathological changes):

1. Clinical signs consistent with ISAV infection, including 2 or more of the following: inappetance, reduction in feed conversion ratio, abnormal positioning in the water column, finfish located near the sides of the holding unit and/or the bottom of the holding unit, ocular hemorrhage, exophthalmia, anemia (pale gills is the most prominent sign), abdominal distension, ecchymotic and petechial hemorrhages present on the ventral aspect of the finfish.
2. Clinical chemistry: hematocrit < 30 (< 10 in severe cases), CBC indicates degenerate and vacuolised erythrocytes, fragmentation of erythrocytes, erythroblasts with irregular nuclear shape, anemia, lymphopenia and thrombocytopenia.
3. Gross pathology consistent with ISAV infection, including: hemorrhages in visceral and parietal peritoneum, swollen and dark (diffuse or focal) kidney, liver and spleen, thin fibrinous layer on the liver, petechial hemorrhages in visceral fat, hemorrhages in pyloric cecae and intestines, dark redness of the intestinal wall mucosa in the blind sacs, mid- and hind-gut without blood in the gut lumen; petechial hemorrhages in the skeletal muscle, edema of the swim bladder, hemorrhage and edema in scale pockets, pale heart, ascites (serous to serosanguinous fluid), serous to serosanguinous pericardial fluid, and no feed in the digestive tract.
4. Histopathology[5] consistent with ISAV infection: vacuolation and nuclear fragmentation of erythrocyes (noted especially during the acute phase of the disease), focal areas of congestion and erythrocytic thrombi in gills, multi-focal to confluent hemorrhagic hepatic necrosis with little evidence of inflammation, focal to multi-focal congestion in dilated hepatic sinusoids, congestion and hemorrhage in intestinal lamina propria, congestion in spleen with erythrophagocytosis, and renal multifocal to diffuse interstitial hemorrhage, tubular degeneration and necrosis, and congestion in glomeruli.

[5] NOTE: Clinical chemistry, gross pathology and histopathology will not be done by NAAHLS, but may be done by other laboratories during the course of an investigation of a disease outbreak. This information should be recorded on the AquaPIQ.

1.7.2 For the purpose of confirmation of the presence of ISAV before initiation of a disease control and response measures (for example, destruction, disposal, cleaning and disinfection, vaccination and surveillance):

ISAV-infected finfish or finfish population

A NAAHLS laboratory or other CFIA-approved laboratory reports, out of the group of finfish tested, at least one positive result for ISAV by virus isolation and identification of the virus by RT-PCR OR 2 positive results using 2 independent tests. Genotyping of the virus strain will be done in all or a subset of positive results. Genotyping may assist in identifying the index and secondary cases and aid in confirmation of non-culturable strains of ISAV.

ISAV-infected watershed (for wild finfish populations residing in fresh water)

A watershed will be considered infected with ISAV if BOTH of the following conditions are met:

Condition 1: laboratory evidence is met if at least one finfish collected from a population of wild finfish tests positive for ISAV.

Condition 2: field evidence of ISAV establishment, is met by one or more of the following:

(a) a second positive finfish originates from a separate species or sampling event of wild finfish, OR

(b) gross pathology and/or histopathology (per licensed pathologist) is consistent with ISAV in at least 1 test-positive finfish.

If finfish are found to be infected, then the water in which they reside is contaminated with ISAV.

NOTE: If a barrier, such as a dam, exists on a natural water course, populations of finfish and water on the other side of the dam from infected populations may not necessarily be infected with ISAV nor is the downstream water necessarily contaminated with ISAV. CFIA will assess the likelihood of infection during an analysis for zonation.

1.8 Laboratories

NAAHLS Reference Laboratory for ISAV

Gulf Fisheries Centre (GFC)
Fisheries and Oceans Canada
343 Université Avenue
PO Box 5030
Moncton, NB, E1C 9B6

Contact Name: Nellie Gagné
Telephone: 506-851-2079
Fax: 506-851-2079

1.9 Definitions

Analytic Sensitivity means the smallest concentration of a substance that can be reliably measured by a particular analytical method.

Analytic Specificity means the ability of the test to detect only the substance of interest.

Biological Vector is an organism, usually an arthropod, in whose body the pathogen develops or multiplies before becoming infective for the recipient individual. The biological vector is an **Intermediate Host** for the pathogen; the aquatic animal is the **Definitive Host**.

Clinical Sensitivity means the proportion of the truly infected population which tests positive. Synonym: **Diagnostic Sensitivity**.

Clinical Specificity means the conditional probability that a person or animal not having the disease will be correctly identified by a test. Synonym: **Diagnostic Specificity**.

Communicable Disease means an infectious or contagious disease.

Control Area is declared by the **Minister**. The Minister may describe the area and identify the disease or toxic substance that is believed to exist there. The Minister may take all reasonable measures consistent with public safety to remedy any dangerous condition or mitigate any danger to life, health, property or the environment that results, or may reasonably be expected to result, from the existence of a disease or toxic substance in a Control Area.

Cultured Finfish means one or more finfish that are being kept.

Definitive Host means the host in which a parasite reproduces sexually. Synonyms: Final Host, Primary Host.

Diadromous means finfish that spend part of their life cycle in seawater and part in freshwater.

Diagnostic Sensitivity: see **Clinical Sensitivity**.

Diagnostic Specificity: see **Clinical Specificity**.

Disease Freedom is a designation applied to zones or compartments that can demonstrate, with an accepted statistical level of confidence, a negligible likelihood of the presence of a certain pathogen.

Effluent water means water in which aquatic animals have resided that is leaving a premises or a defined natural body of water.

Egg Surface-Associated Transmission means the transfer of pathogens to hatchlings through the contamination of the surface of the eggs. Egg surfaces can become contaminated by sexual fluids, skin mucus (through the action of 'stripping'), or water. Cf: **Vertical Transmission**.

Enzootic means a disease in animals that is prevalent in a particular locality. Synonym: endemic, although this term usually applies to human populations.

Epidemiological Unit means a group of animals in a defined location that share approximately the same risk of exposure to a disease agent. This may be because they share a common aquatic environment (eg. fish in a pond, caged fish in a lake), or because management practices make it likely that a disease agent in one group of animals would quickly spread to other animals (eg. all the ponds on a farm, all the ponds in a village system).

Epizootic means attacking many animals in any region at the same time; widely diffused and rapidly spreading. A disease of high morbidity with is only occasionally present in an animal community. Synonym: epidemic, although this term usually applies to human populations.

Finfish means any cold-blooded aquatic vertebrate possessing fins and gills.

Host is an animal or plant that harbours and provides sustenance to another organism.

Independent Tests

Infected Place means a written declaration by an inspector or officer designated under the *Health of Animals Act*. It is issued when an inspector or officer suspects or determines that a disease or toxic substance exists in a place and is of the opinion that it could spread or that animals of things entering the place could become affected or contaminated by it.

Infectiousness means the state or quality of the ability of a pathogen to invade and multiply within an animal.

Influent water means water, intended to come into contact with aquatic animals, that is entering a premises or a defined natural body of water.

Intermediate Host means the organism that is infected by a **Parasite** prior to infecting another Intermediate Host or the **Definitive Host**.

Mechanical Vector means a living organism, animal or person that serves to passively transmit the pathogen to a **Susceptible Species** of aquatic animal but is not infected by the pathogen (and therefore cannot actively shed the pathogen).

Minister means the Minister of Agriculture and Agri-Food (Canada).

Outbreak means an increase in mortality or disease above what is expected in a small localized population of aquatic animals, such as occurs on an aquaculture site or in a lake.

Population means a group of **Epidemiological Units** sharing at least one common defined characteristic.

Premises means a place where aquatic animals are kept.

Quarantine means an order communicated by personal delivery by an inspector designated under the *Health of Animals Act*. A quarantine can be issued for a disease agent, animal or thing. The notice may specify the manner, condition, place or places and time of quarantine necessary to prevent the spread of the **Communicable Disease**.

Surveillance means a systematic series of investigations of a given population of aquatic animals to detect the occurrence of disease for control purposes, and which may involve testing samples from that population.

Susceptible Species means a species of aquatic animal in which infection or infestation has been demonstrated by natural cases or by experimental exposures to the disease agent that mimics the natural pathways for infection or infestation. The disease agent may not be overtly pathogenic for the species. This includes animals denoted as 'carriers' in infectious disease terminology. Carrier means an aquatic animal of a susceptible species that shows no clinical signs of disease but harbors the infectious agent of disease and is capable of transmitting the agent to others because of active shedding of the disease agent. Shedding can be continuous or intermittent. For regulatory purposes, a carrier is considered a Susceptible Species.

Vaccination means the protocol used to administer the vaccine to an aquatic animal or aquatic animal population so that the aquatic animal or population is partially or fully protected from a particular disease for a specified period of time.

Vaccine means a preparation or adjuvanted preparation of killed micro-organisms; living attenuated, fully virulent, or related nonvirulent micro-organisms; or parts of micro- or macro-organisms that are administered to produce or increase immunity to a particular disease.

Vector means an animal that has the potential to transmit a disease, directly or indirectly, from one animal or its excreta to another animal (*Health of Animals Act* (Canada)). This category includes species not listed on the Susceptible Species list. See **Biological Vector** and **Mechanical Vector**.

Wild Finfish means one or more finfish living in natural water bodies (eg. lakes or oceans) or drainage channels (eg. drains created under the *Drainage Act* (ON)) that are not considered a part of a premises, ie. they are not being kept.

1.10 Abbreviations

AAHD	Aquatic Animal Health Division
AAHFP	Aquatic Animal Health Functional Plan
CBC	Complete Blood Count
CFIA	Canadian Food Inspection Agency

gm	gram
HPR	Highly Polymorphic Region
hr	hour
ISA	Infectious Salmon Anaemia
ISAV	Infectious Salmon Anaemia Virus
ISAV HC	Infectious Salmon Anaemia Virus Hazard Characterization
ISAV HSP	Infectious Salmon Anaemia Virus Hazard Specific Plan
min	minute
MSDS	Material Safety Data Sheet
NAAHLS	National Aquatic Animal Health Laboratory System
nm	nanometre
pfu	plaque forming unit
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
TRO	Total Residual Oxidant

2.0 Policy Statements

These policy statements apply to cultured finfish only.

1. All strains of ISAV are subject to disease control and response activities.

2. A disease response to a notification of ISAV is likely to be launched when:

   a) An ISAV incursion occurs outside of its known infected areas, OR

   b) An ISAV incursion occurs within their known areas if:
   - the incursion is in a species of cultured finfish not on the susceptible species list (not listed in the Tables or is highlighted in blue in the Tables in section **3.2.1 Susceptible species**);
   - the incursion occurs in a CFIA-approved compartment.

Descriptions of known infected areas in Canada for ISAV are available from your Area Program Specialist (Aquatic) or:

National Manager, Disease Control and Contingency Planning
Aquatic Animal Health Division
Canadian Food Inspection Agency
Email: NAAHPPNSAA@inspection.gc.ca
Tel: 613-773-7428
Fax: 613-773-7567

3. ISAV is not zoonotic, therefore additional requirements for human health and food safety do not need to be considered during disease response.

4. The Quarantine Order is the preferred option to prevent disease spread until ISAV is confirmed.

5. An Infected Place declaration is the preferred option once the decision to depopulate and re-establish disease freedom is made.
    - However, further consideration of the following is required before an Infected Place declaration is issued for premises located in the marine environment:
        i. probability of achieving long-term disease-freedom status

6. One or more Quarantine Orders are issued in all incursions on premises where the probability of infection is considered high (> 50%) (HIGH RISK[6]).

7. When issuing a Quarantine Order, the following sources of disease spread should be considered for inclusion on the Order: aquatic animals, water, aquatic animal feed, fomites and vectors
    a. If the source of disease (for example: water) cannot be adequately controlled, it will not be described in the Quarantine Order
        i. Spread of the disease agent from effluent water cannot be controlled unless
            - volumes are small and can be released onto the ground in situations where contamination of ground water wells that serve as a source of influent water is negligible OR
            - the effluent can be treated using physical or chemical means, OR
            - the influent water is turned off (for example, when depopulation of all susceptible species will be carried out) and the effluent water is not permitted to leave the quarantined place (for example, the drains are blocked).
    b. Finfish species that are not confirmed as susceptible (see **3.2.1 Susceptible species**) can also be quarantined
    c. Mollusc or crustacean species that are co-cultured on the premises can also be quarantined (eg. integrated multitrophic aquaculture) if they serve as mechanical vectors.

7. One or more Quarantine Orders are issued in all incursions on premises where the probability of infection is considered low (< 50%) (LOW RISK[7]) IF movements are planned that increase the risk of disease spread, otherwise the issuance of Quarantine Orders is at the discretion of the Veterinary Inspector.

[6] HIGH RISK is the term used in the Animal Health Functional Plan. The determination of risk requires knowledge of the probability of the risk occurring (in this case, the risk is 'infection') and the magnitude of the consequences. The Aquatic Animal Health Functional Plan speaks in terms of 'probability of infection' because all the regulated aquatic animal diseases are highly infectious and consequences are severe for Canada. In reality, the clinical probability of infection is all that needs to be determined during an inspection.

[7] LOW RISK is the term used in the Animal Health Functional Plan. The determination of risk requires knowledge of the probability of the risk occurring (in this case, the risk is 'infection') and the magnitude of the consequences. The Aquatic Animal Health Functional Plan speaks in terms of 'probability of infection' because all the regulated aquatic animal diseases are highly infectious and consequences are severe for Canada. In reality, the clinical probability of infection is all that needs to be determined during an inspection.

8. CFIA Veterinary Inspectors and Inspectors are expected to follow any CFIA-specified biosecurity measures and premises-specific biosecurity measures when inspecting the quarantined area.

9. An Infected Place declaration should only be used when eradication will be carried out to achieve a disease-freedom status.
    a. An Infected Place declaration is made after ISAV is confirmed by NAAHLS or a CFIA-approved laboratory and the decision has been made to depopulate.
    b. A further declaration of up to 5 km of the limits of the premises under the Infected Place declaration may only be beneficial to prevent spread
    - if another premises with aquatic animals is located within 5 km of the Infected Place in the same watershed (contact your Area Program Specialist (Aquatic) for advice) and depopulation is planned, OR
    - for premises located in the marine environment in order to control vessel traffic[8]
    c. CFIA Veterinary Inspectors and Inspectors are expected to follow any CFIA-specified biosecurity measures and premises-specific biosecurity measures when inspecting the quarantine area(s) or Infected Place.
    d. People themselves (other than the clothes they are wearing) can serve as mechanical vectors for spread of ISAV to susceptible populations of finfish (eg. hands, face, hair). Therefore, the owner/operator of the premises should institute biosecurity measures in and out of the places named in the Quarantine Order or Infected Place declaration for control of spread of ISAV by people.

10. When surveillance or diagnostic testing is employed as a disease control measure, finfish species that are not confirmed as susceptible (see **3.2.1 Susceptible species**) can also be tested.

11. Depopulation should begin within 24 hours of declaration of an Infected Place
    - Depopulation should proceed as quickly as possible if the likelihood of spread from the premises is not negligible.
    - Pre-emptive slaughter (harvest for food) and/or depopulation over time can be considered on Infected Places where the likelihood of spread from the premises is negligible
        i. Quarantine Orders can be used to control ISAV in specific populations of finfish on an Infected Place, for example, those that are not in the immediate plan for destruction and disposal but their health status requires further monitoring.
    - Both susceptible species and vectors should be considered for depopulation (eg. mussels grown in an integrated multitrophic aquaculture system).

12. ISAV-exposed finfish can be processed for human consumption only if the risk of spread of ISAV into a non-infected area during the transport and slaughtering process will be negligible.

[8] A formal agreement with Transport Canada may be required to allow this further declaration. Contact your Area Program Specialist (Aquatic) for advice on this matter prior to issuing the Infected Place declaration.

13. All movements, including removal from quarantine for sale or transfer of ownership of infected finfish, germplasm, carcasses, offal and parts thereof must be completed under a license:
    - The license must list conditions to control the risk of disease spread to negligible.

14. Cleaning and disinfection must occur after depopulation is complete. Cleaning and disinfection may also occur after each or after a defined number of depopulations if complete depopulation will occur over an extended period of time.

15. Disinfection must result in total inactivation of ISAV:
    - Canadian-approved disinfectants for ISAV should be the first choice for disinfection
    - Decontamination must take into account any federal and provincial laws that regulate chemical sale and use.
    - Disposal of disinfectants should consider the safety of the environment and follow federal and provincial legislation regarding disposal of chemicals.

16. The minimum fallow period should be at least two times the estimated incubation period. A shorter fallow period can be used when the premises has a negligible risk of ISAV introduction and cleaning and disinfection is considered effective.

17. Vaccination (increasing the resistance of susceptible finfish) can be considered as a disease control measure for ISAV in situations where trade is not affected. A commercial vaccine against ISAV is available in Canada, but knowledge of response to vaccination against ISAV is not extensive (vaccination may not prevent infection with shedding or an outbreak).

18. Inactivation of ISAV in/on germplasm, carcasses and offal is required if they are destined for the following end uses: fertilizer, aquatic animal feed, and bait. Inactivation of ISAV can be achieved by exposure of the tissue to high temperatures ($> 60^{\circ}C$ for 10 min) or low pH conditions ($pH < 3.5$ for 8 hr).

19. Declaration of a Control Area can be considered when:
    - Multiple Infected Places have been or will be declared and the declarations have not occurred or will not occur in a known infected area for ISAV;
    - The probability of eradication of ISAV within the Control Area is > 50%.

20. The boundaries of a Control Area should consider watershed delineations if freshwater systems are part of the Control Area, and a tidal excursion determination method if marine systems are part of the Control Area.

21. Zoning within a Control Area should consider watershed delineations if surface water is or is likely to be contaminated or a tidal excursion or some other dispersal determination method if marine systems are part of the Control Area.

22. If a Control Area cannot be revoked because disease control and response activities could not eliminate the pathogen, then re-evaluation of the zonation of an Eradication Area will be conducted by AAHD Disease Control and Contingency Planning.

23. A Control Area can be revoked once the revised zonation of the Eradication Area has been declared.

These policy statements apply to wild finfish populations.

24. When ISAV is detected in wild finfish populations, CFIA may conduct a tracing function to identify exposed premises with cultured aquatic animals or other exposed wild finfish populations.

25. Other disease control activities that may be initiated include surveillance of wild finfish populations (coordinated through DFO) and movement controls of harvested wild finfish.

26. Decisions about disease response activities involving wild finfish populations, including cultured finfish whose end use involves placement into natural bodies of water, will include the government that has jurisdiction over the natural resource.

3.0 Technical Aspects of ISAV and ISA in Relation to Disease Control and Response Planning and Delivery

This section provides a summary of the details available in the Infectious Salmon Anaemia Virus Hazard Characterization, Version 1.0 (Link or RDIMS # to be added upon document approval).

3.1 The disease agent, ISAV

3.1.1 Family/genus and classification

Infectious Salmon Anaemia Virus (ISAV) is a member of the family Orthomyxoviridae, in the genus Isavirus. The 2 main lineages of ISAV are the European genotype and the North American genotype.

There are virulence and antigenic differences of strains associated with these genotypic changes, but these are not yet well defined. HRP0 is considered a non-pathogenic strain of ISAV since detections in populations of Atlantic salmon have not been associated with mortality. This strain has only been detected using PCR methodology; the virus does not grow, or at least, cause obvious cytopathic effects in cell cultures.

3.1.2 Distribution in Canada

ISAV has been found in Atlantic salmon cultured in New Brunswick in the Bay of Fundy. The last detection of a pathogenic strain of ISAV occurred in 2007. Since then, there have been periodic detections of a non-pathogenic strain of ISAV (called HPR0).

3.1.3 Basic characteristics of ISAV

ISAV is an enveloped virus, with surface projections associated with hemagglutinin (receptor binding), receptor destroying and fusion activity. The virus is approximately 100 to 130 nm in diameter and the projections are 10 nm in length. The virus has a genome consisting of 8 single stranded RNA segments. As with other members of the Orthomyxovirus family, re-assortment of gene segments occurs frequently and may contribute to the emergence of new strains.

3.1.4 Survival in the environment

ISAV survival in aquatic environments has been studied in the laboratory. ISAV has the potential to survive for weeks at low temperatures; virus was still infective in cell culture when stored at 4°C sterile seawater for 105 days. At 15°C, ISAV was still infectious at 21 days.

3.2 The disease, ISA

3.2.1 Susceptible species

Natural outbreaks of ISA have been observed in cultured Atlantic salmon and Rainbow trout.

List of species susceptible to ISAV that occur in the natural environment in Canada. Finfish may have several common names but only one common name is being referred to in this list. This list includes carrier species of molluscs. M = marine life cycle; FW = freshwater life cycle; D = diadromous life cycle; the life cycle occurs in both marine and freshwater.
Note: Species coloured in blue have not been confirmed as susceptible to ISAV, but there is some evidence that they can be naturally infected with VHSV.

Scientific Name	Common Name	Life Cycle (M, FW, or D)
Alosa pseudoharengus	Alewife	M
Clupea harengus	Atlantic herring	M
Gadus morhua	Atlantic cod	M
Oncorhynchus kisutch	Coho salmon	D
Oncorhynchus mykiss	Rainbow trout	D
Pollachius virens	Pollack	M
Salmo salar	Atlantic salmon	D
Salmo trutta	Sea trout	D

3.2.2 Infectiousness

The infectiousness of the various strains of ISAV has not been well documented but there is evidence that infectiousness of strains does vary. For example, an HPR4 strain caused more much more rapid mortality than an HPR5 strain; both were isolated from cultured Atlantic salmon in NB.

Strains that are characterized as HRP0 have not been associated with clinical signs.

To date, reported virulence factors include alterations in both segment 5 and segment 6. More specifically, it has been reported that virulent strains exhibit 4 to 22 amino acid deletions in the highly polymorphic region (HPR) of the hemagglutinin-esterase gene (segment 6 of the genome). In addition, these strains also exhibit an insertion of a short amino acid sequence and a point mutation at position 266 in the fusion protein coding sequence (segment 5 of the genome). Additional research is required in order to identify other genotypic differences which may be attributed to ISA virulence. Research has not demonstrated whether an ISA strain which contains the full-HPR sequence (known as HPR0) is avirulent and whether ISA HPR0 could mutate into a more virulent strain.

The strongest predictors of new ISA outbreaks includes: proximity to premises with clinically infected fish, previous history of ISA, lack of fallowing between year classes, and use of harvest vessels that do not practice biocontainment.

3.2.3 Affected life stages of finfish

ISAV will infect and cause disease in all life stages of finfish, except eggs. There is no strong evidence to indicate vertical transmission. However, eggs can be surface-contaminated with ISAV, therefore all life stages and/or age groups must be considered in developing biosecurity procedures, and diagnostic and diseases freedom testing requirements.

3.2.4 Prevalence

An estimate of prevalence is needed to calculate samples sizes for fish that are sick and for fish that appear healthy. In an outbreak on any given premises, even those consisting of net-pens, prevalence between holding units can vary widely.The prevalence within a population of morbid finfish will likely be > 30%. In healthy-appearing populations of finfish, the prevalence can vary substantially, from 0% to 100%; in such populations, sampling should be conducted at an assumed prevalence of 2%.

Based on current knowledge, the prevalence within a population of finfish depends on species susceptibility, age or size of the fish, exposure dose, water temperature and the stage of disease progression in the population. It is likely that density plays a role in affecting within population prevalence but this factor has not been systematically studied. Fish management factors, such as nutrition and handling, also play a role but are only documented anecdotally.

Virulence also plays a role since less virulent strains result in lower within fish viral titres and less fish infected within the population. For the HPR0 strain, within fish viral titres are low but the prevalence within the population can approach 100%.

3.2.5 Modes of transmission

Disease spread is horizontal and indirect through contaminated water. Water can be contaminated by infected animals that are shedding ISAV, and by contaminated fomites or

mechanical vectors. Spread through direct contact with infected fish has not been systematically demonstrated.

A biological vector is not required for completion of the life cycle of this virus.

The portals of entry and routes of shedding have not been established.

There is no evidence for vertical transmission through infected germplasm. However, egg-surface associated transmission may occur as ovarian fluid can be contaminated with the virus. The contamination of milt has not been demonstrated.

3.2.6 Incubation period

Experimental challenge studies report an incubation period of 17 to 23 days at 12˚C and high exposure doses. In most of the experimental bath or cohabitation challenge studies, the incubation period was measured as the time from exposure to first mortality, rather than to the appearance of clinical signs.

The incubation period for ISA varies with viral dose during exposure, water temperature, and age of the fish. Virulence may also play a role but has not been systematically investigated. The higher the exposure dose, the warmer the water temperature and the younger the fish, the shorter the incubation period.

3.2.7 Attack rates and mortality curves

Morbidity and mortality can vary greatly both within and between different net-pens on an aquaculture farm and between farms.

Morbidity and mortality within a net-pen may start at very low levels. Typically, during an outbreak, daily mortality ranges from 0.5% to 1% in affected cages.

Cumulative mortality may range from 15-100%.

During the progression of a natural outbreak in cultured Atlantic salmon, mortality increases and peaks in the winter and early summer.

3.2.8 Persistence of infection

In Atlantic salmon, persistent long-term infection with or without shedding has not been documented.

3.2.9 Aquatic animal feed as a source of ISAV

ISAV will not survive a heat treatment process if it is used to manufacture feeds. However, the possibility of cross-contamination needs to be assessed in individual aquatic animal feed manufacturing plants.

All other types of feeds, such as live feeds and moist feed, if infected with ISAV, pose a risk for transmission of the disease.

3.2.10 Fomites as a source of ISAV

Fomites can serve as transmitters of ISAV. Large point sources of ISAV include vessels that transport live or dead fish and processing plants.

3.2.10 Mechanical vectors as a source of ISAV

Birds may serve as mechanical vectors but the significance is unknown. The role of other terrestrial or aquatic wildlife, insects, and parasites has not been determined.

3.2.12 Diagnosis of ISA

NAAHLS will confirm the diagnosis of ISAV using cell culture and identification of the virus using PCR OR application of 2 independent test methodologies.

When clinical signs are present, other Reportable diseases that could be included in the differential list are: Viral haemorrhagic septicaemia, Infectious haemorrhagic necrosis, and Infectious pancreatic necrosis. Annually notifiable diseases that should be differentiated from ISA are Enteric red mouth disease and Furunculosis.

3.2.13 Diagnostic test characteristics

Contact NAAHLS for more specific information on analytical and clinical sensitivity and specificity estimates of the validated tests.

The number of fish to sample, based on assumed within population prevalence and diagnostic test characteristics, can be viewed in Table 1 of the procedure *Inspection Phase/Action Phase of Disease Response: Sampling of Cultured Finfish* (RDIMS # 2230752).

More precise sample size calculations can be performed using @RISK, FreeCalc2, or another computer program.

3.2.14 Tissues to sample

ISAV has an affinity for the endothelial cells in many organs. Tissues with high numbers of endothelial cells include heart, kidney and spleen. Blood and ovarian fluid may be taken for non-lethal sampling.

In order to detect HRP0 (non-culturable form of ISAV), gill specimens must be tested.

Pooling of small finfish and specimens can be done when fish are sick; up to 5 fish per pool is acceptable. Finfish and/or specimens should not be pooled when apparently healthy populations

of finfish are sampled. Pooling of samples/specimens when within finfish and between finfish prevalences are low could lead to false negative test results.

3.2.15 Vaccines and vaccination

Current vaccines may not offer adequate protection in the field. The vaccines, which are inactivated whole virus vaccines, may not protect against infection, including with the HPR0 strain.

One vaccine for ISAV is approved in Canada: Forte V1 – Infectious Salmon Anaemia Virus Vaccine, Killed Virus, Aeromonas salmonicida, Vibrio anguillarum-ordalii-salmonicida Bacterin, Novartis (Aqua Health).

3.2.16 Treatments

No treatments are known at this time.

3.2.17 Disinfectants and disinfection

The following items can be disinfected: finfish eggs, water, and fomites, including the bottom of drained ponds.

ISAV is an enveloped virus, and relatively large; properly applied disinfection programs (according to manufacturer's directions) are likely to be successful with most disinfectants.

Methods used to inactivate ISAV are heat (minimum of > 50˚C for 2 min), UV (> 25 mJ/cm²; 254 nm), ozonation (8 mg/L/min for 4 min), and chemicals (iodine, hypochlorites (100 ppm for 15 minutes), acids (pH < 4 for 30 min) and lye (caustic soda) (pH > 12 for 7 hr).

NOTE: The use of alcohol-based hand gels will not result in effective disinfection; gloves should be worn when handling infected or contaminated animals or things.

NOTE: Hypochlorites are extremely toxic for finfish. Thorough rinsing and a fallow period post-disinfection are required prior to restocking with finfish (for example, holding units) or using in the presence of finfish (for example, dip nets).

NOTE: Effective disinfection of finfish eggs that have been exposed to very high titres of ISAV in the ovarian fluid has not been evaluated.

<u>Canadian-approved disinfectants for use in aquatic systems</u>

These disinfectants should be first choice when planning disinfection:

Ovadine™ is a polyvinylpyrrolidone-iodine complex approved in Canada (DIN 02305712) for the disinfection of salmonid eggs. Ovadine™ is considered effective against surface contamination of eggs by ISAV when used according to manufacturer's directions. The MSDS

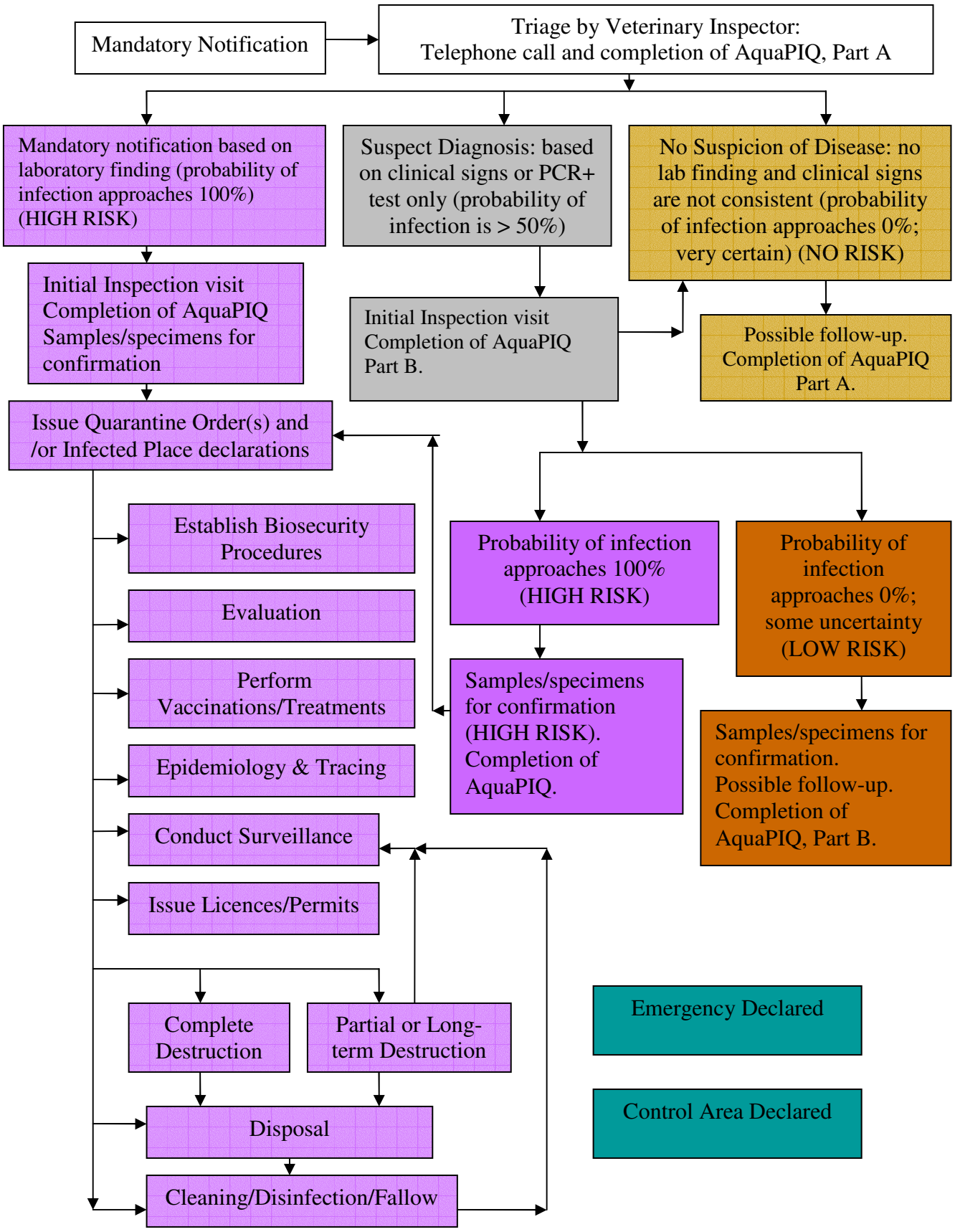
for Ovadine™ is available from Syndel (http://www.syndel.com/Assets/File/Ovadine_MSDS.pdf).

Virkon® Aquatic is a potassium monopersulfate approved for use in Canada (DIN 02276356) and is labeled for disinfection against ISAV. It acts as an acid. It can be used to clean equipment, vehicles, and instruments. Virkon® Aquatic can also be used as a footbath. Manufacturer's directions should be followed. The MSDS for Virkon® Aquatic is available from Western Chemical Inc. The disinfecting protocol is 1:100 dilution at 20˚C for 30 minutes (http://www.wchemical.com/Assets/File/virkonAquatic_MSDS.pdf).

Other disinfectants not approved for use in aquatic systems (ie. when fish are present)

Peroxigard™ is available from Bayer Healthcare, and is virucidal for enveloped viruses.

Appendix A: Process flow for response to an incursion of a Reportable or Immediately Notifiable aquatic animal disease.


Mandatory Notification
Triage by Veterinary Inspector:
Telephone call and completion of AquaPIQ, Part A
Mandatory notification based on laboratory finding (probability of infection approaches 100%) (HIGH RISK)
Suspect Diagnosis: based on clinical signs or PCR+ test only (probability of infection is > 50%)
No Suspicion of Disease: no lab finding and clinical signs are not consistent (probability of infection approaches 0%; very certain) (NO RISK)
Initial Inspection visit
Completion of AquaPIQ
Samples/specimens for confirmation
Initial Inspection visit
Completion of AquaPIQ
Part B.
Possible follow-up.
Completion of AquaPIQ
Part A.
Issue Quarantine Order(s) and /or Infected Place declarations
Establish Biosecurity Procedures
Evaluation
Perform Vaccinations/Treatments
Epidemiology & Tracing
Conduct Surveillance
Issue Licences/Permits
Probability of infection approaches 100% (HIGH RISK)
Probability of infection approaches 0%; some uncertainty (LOW RISK)
Samples/specimens for confirmation (HIGH RISK). Completion of AquaPIQ.
Samples/specimens for confirmation.
Possible follow-up.
Completion of AquaPIQ, Part B.
Complete Destruction
Partial or Long-term Destruction
Disposal
Cleaning/Disinfection/Fallow
Emergency Declared
Control Area Declared