Maine
Infectious Salmon Anemia Virus Control Program Standards

USDA APHIS Veterinary Services

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Executive Summary

The United States Department of Agriculture, (USDA), Animal and Plant Health Inspection Services, (APHIS), Veterinary Services, (VS) *Infectious Salmon Anemia Virus Control Program Standards*, hereafter referred to as the Standards, establish recommended procedures for the prevention and containment of Infectious Salmon Anemia virus (ISAV) in farm-raised Atlantic salmon (*Salmo salar*) in Maine. The Standards provide guidelines for producers, APHIS-accredited veterinarians, other fish health personnel, laboratory personnel, and regulators. The eligibility of Atlantic salmon producers affected by ISA in Maine to receive indemnity is based on compliance with these Standards, (*See 9 CFR 53.10* for additional details). These Standards were originally written in 2001 by the USDA APHIS VS after numerous meetings, consultations, and discussions with members the Standards Committee of the Maine/USDA APHIS VS ISA Joint Working Group. This is the third revision completed by the *ISA Technical Board*, with input from subject matter experts, to reflect updated information regarding ISAV/ISA transmission, diagnostic tests, control processes, etc. It is understood that these Standards must be open to amendment as science and circumstances evolve.

Introduction and Background

Infectious salmon anemia (ISA) is a disease of Atlantic salmon (*Salmo salar*) caused by infectious salmon anemia virus (ISAV). Detections of the virus, or clinical outbreaks of disease caused by it, are notifiable to the World Organisation for Animal Health (WOAH) by competent authorities worldwide. All non-negative detections of ISAV of any genotype, or outbreaks of ISA in the U.S., are *reportable* to USDA APHIS Veterinary Services as the Federal competent authority for animal health. Detections are also reportable to *Maine officials*.

ISAV is found in farmed and free-ranging susceptible fish species in freshwater and marine settings. While ISA is a disease of Atlantic salmon (*Salmo salar*) (WOAH, 2022), other species in which ISAV detections have been made include rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), and pollock (*Pollachius virens*). ISAV does not affect humans or other mammals and the virus has not been shown to replicate at typical mammalian temperatures (Falk et al, 1997).

Both ISAV infection and any resulting disease are of serious global concern for the farmed Atlantic salmon industry. Outbreaks of ISA have impacted Atlantic salmon production in Norway, Scotland (and other parts of the UK), the Faroe Islands, Maritime Canada, the U.S. (Maine only), and Chile. Hemorrhagic kidney syndrome (HKS) in Atlantic salmon from New Brunswick, Canada was first described in 1998, and appears in retrospect to be the first account of ISA in North America (Mullins et al., 1998). The first isolate of ISAV discovered in Maine in 1999 was determined to be the same strain as the New Brunswick 1998 HKS virus (Bouchard et al., 1999; Blake et al., 1999). Outbreaks of ISA between 1999 and 2003 in Maine and Maritime Canada caused devastating economic losses to the industry in both countries.

Taxonomically, ISAV is the type species of the genus *Isavirus*, in the *Orthomyxoviridae* family (ICTVdB Index of Viruses), which also includes influenza viruses. The genome of ISAV is comprised of eight single-stranded segments of RNA of negative polarity. Virus particles are pleomorphic, enveloped, and 100-130 nm in diameter with 10-12 nm surface projections. ISAV is inactivated by external heat, extreme pH, UV, ozone, and a variety of disinfectants containing chlorine, iodine, or potassium peroxymonosulfate compounds (Falk et al., 1997). Virus persistence is also likely influenced by environmental factors such as temperature, salinity, presence of organic material, presence of other pathogens and exposure to UV radiation (WOAH 2022).

Route of infection and clinical signs

Gill tissue is considered the main route of ISAV entry (Weli et al., 2013), though skin and gastrointestinal tract are also noted (Aamalfot et al, 2015; Rolland and Nylund, 1998a). ISAV infects endothelial cells in blood vessels, kidney, and heart tissues (Aamelfot et al., 2012), resulting in internal and external hemorrhages in clinically affected fish (WOAH 2022).

Morbidity and mortality due to ISAV are highly variable. Cumulative mortality varies greatly from zero to a majority of fish on a given site. Outwardly, affected fish may show few signs of infection or disease until late in the disease course, or may alternatively exhibit a variety of associated signs including pale gills, exophthalmia (with or without hemorrhage), lethargy, petechiae (pinpoint hemorrhages) on the skin, and darkened skin surfaces. Gross internal lesions are also highly variable and may include: 1) fluid accumulation in the peritoneal (ascites) or pericardial spaces, 2) hemorrhagic lesions along the GI tract, mesenteric fat, swim bladder or peritoneum, and 3) hemorrhagic lesions, congestion, swelling or darkening of the liver, spleen and kidney capsule. Microscopic lesions may include congestion and necrosis in the liver, heart, blood vessels, spleen and kidneys. (WOAH, 2022).

Epidemiological factors

Transmission of ISAV is believed to occur principally through indirect horizontal routes (Nylund et al., 2019). Virus is shed into the environment through blood, mucus, feces, urine, skin or decomposing carcasses of infected fish (Vike et al., 2014). Epidemiological investigations suggest the virus also spreads between aquaculture sites via untreated wastes (e.g., offal and blood water)and water from harvest operations and processing plants (Vagsholm et al., 1994), natural circulation of water between infected and uninfected sites (Gustafson et al., 2007a; Mardones et al, 2009; Aldrin et al., 2011), wild fish (McClure et al., 2005), a shared work force (Vagsholm et al., 1994; Hammell and Dohoo, 2005), and shared equipment or gear that have not been properly disinfected at marine sites (Hammell and Dohoo, 2005; Ellis et al., 2006).

An investigation of ISA in first-feed Atlantic salmon fry raised the possibility of infection at very early life stages through contaminated ovarian fluids (Nylund et al., 1999). Though the importance of this pathway is debated (Christiansen et al., 2021), some studies suggest that certain ISAV genotypes may be transmitted vertically (i.e., from parent to offspring) through infected eggs

(Kibenge et al., 2009; Marshall et al., 2014; Vanderstiche et al., 2014, Nylund et al., 2019). Surface disinfection of eggs is highly recommended as a risk mitigation for ISAV.

Arthropods such as sea lice (*Lepeophtheirus salmonis and Caligus sp.*) are considered potential pathogen vectors, but their significance in ISAV transfer remains unclear (Valdes-Donoso et al., 2013, Barker et al., 2019). Secondary effects such as stress or tissue damage may also increase viral susceptibility (Rolland and Nylund, 1998a). Little supporting evidence is available to demonstrate that other marine species act as ISAV vectors or reservoirs capable of infecting cultured Atlantic salmon.

Genomic information

Two distinct genotypes have been identified based upon analysis of sequence data from ISAV segments 2, 5, 6 and 8: Genotype I and Genotype II (Kibenge et al., 2001; Krossoy et al., 2001; Kibenge et al., 2009, Gagne & LeBlanc, 2018). Genotype I, also known as 'European clade', is the most common type in Norway, Scotland, Shetland Islands and the Faroe Islands, although this genotype also occurs in North America. Genotype II, also known as 'North American clade', is predominantly associated with Atlantic salmon in North America. Studies of viral genomes from Norway, Scotland, and Canada indicate that these two subtypes appear to have diverged more than 100 years ago (Krossoy et al., 2001).

Phylogenetic studies of surface glycoprotein gene sequences encoded on ISAV segments 5 and 6 further subdivide the European clade into three geno-groups, EU-G1, EU-G2 and EU-G3 (Nylund et al., 2003; Devold et al., 2006; Kibenge et al., 2009). Variability of the North American clade appears less substantive, so it has not been similarly subdivided.

Additionally, genetic variation among isolates has been investigated for use as markers of virulence and for tracing between farms and regions (Krossoy et al., 2001a), with a focus on gene segments encoding surface hemagglutinin-esterase (HE, segment 6) and fusion (segment 5) proteins (Nylund et al., 2003; Godoy et al., 2013). Currently, the greatest genetic variability is found in a region of the HE gene termed the highly polymorphic region (HPR), encoding the stem of the HE protein. Many amino acid patterns have been described for this HPR (Aamelfot et al., 2014). To date, all clinical ISAV variants include gaps in the HPR sequence, hypothesized to arise through deletions from a full-length precursor gene termed HPR0 (EFSA, 2012). These variants, commonly associated with clinical outbreaks, are loosely referred to as 'HPR-deleted' (WOAH 2022). To date, HPR0 variants

have mostly proven non-culturable and have never been associated with disease (Ritchie et al., 2001; Kibenge et al., 2009; Godoy et al., 2013). While isolates with the HPR0 signature display tissue tropism for gill epithelium (Christiansen et al., 2011), HPR0 infection does not appear to cause ISA clinical signs or increased mortality (Christiansen et al., 2011; Godoy et al., 2013). HPR0 and HPR-deleted variants are found in both the European and North American clades.

HPR0 variants occur in salmon production regions globally (Kibenge et al., 2009; Christiansen et al., 2011; Lyngstad et al., 2011; Vanderstiche et al., 2014). Detections of HPR0 variants have been documented antecedent to, concurrent with, or subsequent to detections of HPR-deleted variants. Additionally, co-detections of different HPR-deleted variants (Aldrin et al., 2011), or detections of both deleted and non-deleted variants (Gustafson et al., 2008; Kibenge et al., 2009; Cardenas et al., 2014) have at times been reported from a single fish or on a single site. Field research has also shown phylogenetic and temporal relationships between HPR0 and virulent variants in some locations (Lyngstad et al., 2007, 2011; Godoy et al., 2013). However, these few findings, and relatively ubiquitous occurrence of HPR0 variants, suggest that the emergence of HPR-deleted from non-deleted virus is likely a low probability event but not negligible (Lyngstad et al., 2012, Cardenas et al., 2014, LeBlanc et al., 2018, Nylund et al., 2019). An alternative hypothesis suggests HPR0 could instead, or also, derive from insertions and attenuation of HPR-deleted viruses (Kibenge et al., 2007; Kibenge et al., 2012; Castro-Nallar et al., 2011; LeBlanc et al, 2018; Rimstad & Markussen, 2019). Since 1996, both North American and European HPR0 and HPR-deleted ISAV variants have been detected in New Brunswick and Maine (USDA ISAV Program data; NBDAAF ISAV Program data).

In addition to deletions in the HPR, ISAV virulence appears co-dependent on insertions or mutations in the fusion (F) protein gene (Devold et al., 2006; Markussen, et al., 2008; Cardenas et al., 2014) and possibly other less-studied sections of the ISAV genome (Markussen et al., 2008). Initial studies propose a multi-step evolutionary process, citing transitional stages (HPR-deleted without a fusion protein mutation), wherein an HPR deletion alone may not be enough to infer virulence (Cardenas et al., 2014). Thus, further evaluation of the HE gene (e.g., the 5' end) and other regions of the ISAV genome is advised to best establish relatedness or infer pathways of disease spread (Aldrin et al., 2011; Lyngstad et al., 2011; Godoy et al., 2013).

Management impacts

In December 2001, the US Secretary of Agriculture declared an animal disease emergency in response to ISAV emergence in a highly productive salmon farming region in Maine. In January 2002, APHIS VS and the State of Maine, in collaboration with the local industry, instituted the ISAV Control Program to oversee disease response at affected Atlantic salmon farming sites in Maine. Program standards, eligibility for indemnification of specified losses, harmonization of control actions with neighboring New Brunswick, and strong cooperation (including the implementation of strict biosecurity protocols) from salmon producers in the U.S. and Canada ultimately led to control of ISA disease in the region (Ellis et al., 2006). The last detection of ISA disease in Maine associated with this initial event occurred in February 2006. Various HPR-deleted findings have been reported in Canada (Romero et al, 2022; Gagne & LeBlanc, 2017) and in Maine (USDA ISAV Program data, 2023), since then. Typically, these have been localized and either isolated or responsive to control. ISAV HPR0 has also been periodically detected by RT-PCR at marine and freshwater sites in Maine and Maritime Canada.

Experience with ISA outbreaks at Atlantic salmon marine sites in Europe, North America and Chile indicates that HPR-deleted ISAV genotypes can spread variably from net-pen to net-pen or site to site if outbreaks are not controlled. Onset of disease may be extended by several months in some net-pens and may be influenced by: speed of infected net-pen removal (McClure et al., 2004; Gustafson et la., 2006; Mardones et al., 2013), water temperature and length of time the fish have been in saltwater, fish density (Mardones et al., 2013), vaccination status (Chase-Topping et al, 2021), sea lice, nutrition, environmental or management conditions (Hammell and Dohoo, 2005; Gustafson et al., 2007b), and the immune competency of exposed fish. Coordination of production activities through the hydrographic delineation of management areas, single year-class stocking of sites, synchronized fallowing within management areas, rigorous biosecurity, and movement restrictions have also been deemed important in the resolution of ISA outbreaks in the United States (Ellis et al., 2006) and other countries (Murray et al., 2010; Gustafson et al., 2014; Mardones et al., 2014).

Major ISAV Control Program Components

The ISAV Control Program was implemented in Maine in January 2002 and is a collaboration between the United States Department of Agriculture, Animal and Plant Health Inspection Service Veterinary Services (USDA APHIS VS), the Maine Department of Marine Resources, APHIS-

accredited veterinarians, an APHIS-approved diagnostic laboratory, the National Veterinary Services Laboratories in Ames, IA, and producers of cultured Atlantic salmon.

The ISAV Control Program consists of seven components: surveillance, laboratory testing, disease reporting, disease control and biosecurity, quarantine, depopulation, and indemnity eligibility. Frequent targeted surveillance ensures that ISAV will be quickly detected when present. Testing with robust assays at an APHIS-approved laboratory facilitates a prompt and accurate diagnosis. Reporting procedures ensure that once infected or diseased fish are identified, control measures can proceed rapidly. Disease control practices such as biosecurity measures and integrated pest management can also mitigate pathogen and disease introduction or spread. Prompt depopulation of net-pens infected with disease-causing HPR-deleted ISAV eliminates a continuing transmission risk. Finally, indemnity may provide financial relief to producers while encouraging prompt reporting and compliance with the Standards. Other related aspects of the Program include: the development of action plans, risk identification and mitigation, movement restrictions, selective depopulation, synchronized fallowing, overall management coordination, and shared communications among Maine and New Brunswick marine Atlantic salmon farming operations.

While elimination of the ISA virus from the marine environment may not be realistic because of the complexity of the marine ecosystem, elimination of the disease from aquaculture operations is an achievable goal. Fish health regulatory agencies in other countries, and many Atlantic salmon producers through their Best Management Practices or international certification regimens, have developed similar approaches to disease management for ISA.

The salmonid farming community in Maine and Maritime Canada utilize qualified personnel experienced in all aspects of fish culture, husbandry, and health management. Companies have established comprehensive internal procedures for increased disease surveillance and utilize working relationships with aquaculture veterinarians and diagnostic laboratories to provide further technical expertise. Current state-level requirements in Maine include that imports of salmon be from a source with a Qualified Source/Hatchery designation or a source that has been given special permission from the Commissioner after review by the Maine Aquatic Animal Health Technical Committee with importation into an approved quarantine facility. Transfer from freshwater hatcheries within the State to marine sites requires a permit, current annual facility health inspection, and when applicable, satisfaction of post import screening requirements for a release from quarantine for that which was

imported with special permission from the Commissioner. Annual facility inspection requires screening of all lots of Atlantic salmon, including broodstock, and other fish species present at the freshwater hatchery for viruses by cell culture. For lots which are imported with special permission by the Commissioner, screening requirements may include pre-import and post-import screening requirements for ISAV by PCR. Additional elective diagnostic sampling may also be conducted to meet individual hatchery or marine site management protocols and production requirements.

Producers of farmed Atlantic salmon in Maine have worked, over time, with the Maine Aquaculture Association, academic and private–sector disease researchers, and regulatory agencies to establish biosecurity protocols, best management practices, integrated pest management and bay management approaches for aquaculture operations. These have included the use of risk assessments, biosecurity audits, and the designation of fish health zones. To ensure that strategies for ISA control are consistently implemented before, during, and after any ISA outbreaks, the Maine Department of Marine Resources **(**DMR) established regulations (*Chapter 24.21*) to define fish health zones (also termed bay management areas or restricted areas), require mandatory ISA virus surveillance (through this Program) and reporting, and restrict finfish aquaculture vessel and equipment movements. In addition, these Standards were developed to: specify which testing procedures are necessary to confirm ISA virus; stipulate chain of custody requirements; standardize sampling requirement protocols; identify responsible inspectors; and stipulate consequent actions upon detection of ISA virus.

Since 2002, Maine farmed salmon producers have prioritized single year-class stocking, as well as zoned site management strategies. In view of the uncertainty surrounding potential vertical transmission of ISA virus, Atlantic salmon producers have voluntarily adopted precautionary measures that apply to many areas of fish husbandry to limit the risk of vertical transmission. These measures include broodstock pre-screening for ISA virus, culling of confirmed positive net-pens, and thorough egg disinfection protocols at fertilization and water hardening. Due to shared water resources, as well as potentially shared personnel and equipment by farms raising fish in both Canada (especially New Brunswick) and Maine, the continuous communication, coordination, and harmonization of ISA management programs between the two countries is essential.

Part I. Definitions

Accredited veterinarian: a veterinarian holding a current Maine State veterinary license, who has also fulfilled the current accreditation requirements for Category II as specified by the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA, APHIS) *See Part IIIA 5b*

Action Plan: a document that defines response contingencies for a particular threat such as a pathogen or disease occurrence.

Active marine site: a marine finfish culture site as designated by the Maine DMR, and at which fish are present, located within the coastal waters of the state.

Approved laboratory: a laboratory that is approved to conduct official diagnostic tests for ISA virus by the USDA APHIS laboratory approval processes (Reference VS memorandum 567.2 and *eCFR : 9 CFR 71.22 -- Approval of laboratories to conduct official testing.*)

Assays: specific tests used for the detection of pathogens

Aquaculture Health Staff: USDA APHIS VS Strategy and Policy staff with national oversight of aquaculture animal health

Atlantic salmon: for this document, all strains of *Salmo salar* being raised or maintained under cultivation conditions.

Biosecurity: procedures designed to eliminate or lessen physical, economic, and other losses involving farmed stocks. Among other elements, risks of acquiring or transmitting pathogens are assessed and factored into a comprehensive program involving aspects of site design, stock selection, and husbandry practices, therapeutic agents, veterinary supervision, and many other management variables (See *Appendix A*)

Biosecurity audit: onsite visit to a hatchery, marine site, processing plant, vessel or facility servicing or involved with aquaculture operations to assess biosecurity and/or audit management practices for compliance with recommended or generally accepted biosecurity protocols (*See Appendix A* or *Part III A3*)

Blood-water: water mixed with blood from harvested fish. This may be found in stun or bleed tanks, vessel holds, container boxes, or processing plants.

Broodstock: reproductively mature fish that have been selected or used as a part of a defined breeding program. These fish will be separately valued as 'broodstock' for any indemnification purposes.

Broodstock candidates: a group of animals from which it is anticipated that the final broodstock will be selected. These fish will be separately valued as 'production fish' for any indemnification purposes.

Cell culture: the process by which cells are grown under controlled conditions, generally outside of their natural environment; *See Virus Isolation*

Clinical signs: any visual signs of disease by gross external or internal examination

Depopulation: removal of all fish of a defined fish population within a net-pen, lease site, or other venue with the intent of preventing a disease outbreak and/or minimizing pathogen spread.

Dip net: apparatus for removing fish from the surface water of net-pens.

Disease: a syndrome including clinical signs, impairment, and/or mortality resulting from infection with a pathogen or from other causes such as water quality, environmental factors, nutrition, genetics, etc.

Pathogen of Regulatory Concern: infectious disease agents that have been demonstrated to cause a significant increase in the risk of morbidity and/or mortality among salmonid populations in the State of Maine. Pathogens of Regulatory concern are defined in Maine DMR Chapter 24 Regulations (*See* section 24.21)

DMR: The Maine Department of Marine Resources

Fallow: the status of a site or defined management zone once all animals have been removed and all equipment has been cleaned and disinfected; *See Part IV E*

Fallowing time: the period of time that a previously active site or defined management zone is empty of animals; this period begins after depopulation/harvest and upon the completion of the cleaning and disinfection of all associated enclosures, equipment and other fomites and it ends when restocking begins; *See Part IV E*

Marine Fish Health Zone: defined marine geographic area as designated in Maine DMR Chapter 24 regulations (*See* section 24.01), also termed bay management area

Genomic sequence(ing): to determine the genetic code (nucleotide sequence that encodes amino acids) of a portion (e.g. HPR) or the entirety of the ISAV genome.

Genotype: the particular combination of alleles for a particular gene or locus

Gross Pathology: any visual signs of disease in fish organs or tissues by gross external or internal examination

Harvest: the removal of fish from enclosures, generally for transportation to a processing plant. Removal usually occurs by means of either containment in a vessel hold (live or dead), or by containment in refrigerated boxes after slaughter on the site.

HPR0 ISAV: This lineage is defined by the highly polymorphic region (HPR) of gene segment 6, which encodes the stem of the HE protein of ISAV and is found to be 105 nucleotides which encode 35 amino acids.

HPR-deleted ISAV: This lineage is defined by the highly polymorphic region (HPR) of gene segment 6, which encodes the stem of the HE protein of ISAV and is found to be less than 105 nucleotides which encode less than 35 amino acids.

Indemnity: compensation paid to producers of Atlantic salmon or other cultured susceptible species in exchange for federally mandated depopulation; *See Part IV B*

IFAT: indirect fluorescent antibody test; an assay that incorporates the binding capacity of specific antibodies to selected antigens. The IFAT makes use of a fluorescently tagged secondary antibody that binds to a primary antibody specific to the target antigen or pathogen.

Import: to land on, bring into, or deposit, in any place subject to the jurisdiction of the State of Maine from outside the State of Maine.

Inspection: an on-site visit and/or a sampling of fish, and the resulting laboratory tests and inspection reports conducted by an inspector in accordance with the testing requirements and procedures set forth in these Standards

Inspector: means an APHIS-accredited, licensed veterinarian, an AFS-certified (American Fisheries Society-certified) fish health inspector, or other persons recognized by federal or state agencies with responsibility for fish health or transfer in the state from which the fish or gametes originate; *See Accredited Veterinarian*

ISA: infectious salmon anemia; the clinical disease resulting from infection by a pathogenic genotype of ISA virus

ISAV: Infectious salmon anemia virus, all known variants; may or may not cause disease

ISA Program Categories: staged categories of ISA status; *See Table 3*

ISAV Control Program Veterinarian: USDA APHIS VS Veterinary Medical Officer, assigned by the VS District 1 Director (DD) or New England AVIC, to manage the ISA Program in Maine, and who reports to the District 1 DD or AVIC; aka Program Veterinarian.

ISA Technical Board: a group of four voting members approved by the New England AVIC or delegate; consisting of the USDA ISAV Control Program Veterinarian, one Maine DMR

representative, two industry representatives (as nominated by Atlantic salmon producers), plus a non-voting chairperson selected by the four voting members**.** Each voting member pre-selects a voting proxy in the event they are unavailable at the time of a vote. *See Part II C*

Integrated Pest Management: also known as integrated pest control is a broad-based approach that integrates both chemical and non-chemical practices for economic control of pests. IPM aims to suppress pest populations below the economic injury level.

National Reference Laboratory: USDA APHIS VS National Veterinary Services Laboratories (NVSL) is the reference laboratory for ISAV.

Negative site: an active site testing negative during the preceding two months for any ISAV genotype while involved with active participation in an official surveillance program, including inspection by a veterinarian (*See* ISA Case Definition Category 1 in *Table 3*)

Non-negative test result: A non-negative test result is any result from an official test that cannot be called negative per the NVSL protocol (e.g., positive, suspect, discrepant, or equivocal);

Net-pens: also called "cages" or "pens"; plastic or steel structures of differing sizes and shapes designed to contain variable numbers of fish; most pens are 100m plastic circles.

Non-pathogenic: not known to induce clinical disease

Pathogen: an agent (bacteria, viruses, fungi, parasites, etc.) capable of causing pathological changes in tissues. Not all pathogens cause clinical disease, and not all diseases are caused by pathogens.

Pathogenic: known to induce disease

Primary testing: Screening tests for ISAV, refer to *Table 2*

Processing plant: any facility where Atlantic salmon, or other ISAV-susceptible species, including products such as fillets used for value-added purposes, are taken for processing or rendering into a marketable product.

Producer: an individual or company raising Atlantic salmon, or other ISAV-susceptible species

Quarantine: enforced isolation to prevent the spread of infectious disease; *See Part IV A3*

Restricted Movement Permit: a USDA APHIS Veterinary Services document entitled "Permit for Movement of Restricted Animals" (VS form 1-27) which allows for the secure movement of fish from sites.

Restriction: no movement of live fish is allowed from the site, except for slaughter; and the implementation of a biosecurity program approved by the Maine DMR is required.

RT-PCR: reverse-transcription polymerase chain reaction assay typically targets specific conserved regions of the viral genome for diagnosis; a screening real-time assay targeting segment 8 and a genotyping gel-based assay targeting segment 6 are available for ISAV.

Sampler: an accredited veterinarian (or a person trained and designated by and under the direction of an accredited veterinarian) for the collection and submission of surveillance and diagnostic samples.

Sea lice: copepod arthropods belonging to either the *Lepeophtheirus* or *Caligus* genera.

Secondary testing: Second tier tests for ISAV, refer to *Table 2*

Sequence: See Genomic Sequence(ing)

Single Year-Class Site: an active site containing only one year-class of fish.

Site: a specific area or facility where fish are raised (e.g., sea cages)

Site Identifier: a finfish aquaculture lease site identification number or code assigned by the DMR and unique for each site.

Site veterinarian: a Maine licensed and USDA APHIS-*accredited veterinarian* with fish health responsibility for one or more marine or freshwater sites where Atlantic salmon, or other ISAV-susceptible species, are cultured.

Smolt: the stage at which Atlantic salmon are capable of physically and metabolically transforming to accommodate large osmotic differences as they move from a freshwater to a saltwater habitat.

Surveillance: a program designed to detect or monitor the presence or absence of a pathogen or disease through periodic sampling and testing of fish within sites

Transfer permit: a permit issued by the Maine DMR that authorizes the recipient to transfer live fish, fertilized eggs, or gametes to or from designated geographical area(s) in the coastal waters of Maine during a specified time period. A transfer permit may not be issued until the DMR has reviewed fish health inspection reports.

Vertical transmission: transfer of an infectious agent from one generation to another

Vessel permit: a permit issued by the Maine DMR that authorized the recipient to move a vessel from one bay in Maine to another.

Virus Isolation: *See Cell culture*

Year class (YC): for the ISA program YC is defined as the year of transfer from a hatchery to a marine site (aka a grouping of a population of fish of the same age/hatch year).

Part II. Administrative procedures

A. Supervision of the ISAV Control Program

The USDA APHIS VS District 1 Field Operations (FiOps), located in the New England Area office in Uxbridge MA, has administrative and supervisory responsibility for administering and managing all USDA, APHIS, VS, sector programs in New England, including the ISAV Control Program in Maine. The New England Area Veterinarian in Charge (AVIC) will appoint a VS Veterinary Medical Officer to oversee the ISAV Control Program, designated as the Program Veterinarian. The National Veterinary Services Laboratories (NVSL) located in Ames, IA is the national reference laboratory for ISAV, meaning NVSL is designated as the confirmatory laboratory for WOAH reporting purposes.

ISAV Control Program Veterinarian: The Program Veterinarian is responsible for overall field management and administration of the ISAV Control Program in Maine and reports directly to the New England AVIC. Major duties and responsibilities for this position include but are not limited to: assigning personnel as needed to carry out control program goals; collating surveillance reports from producers, site veterinarians, or the approved laboratories; resolving the accuracy of the respective surveillance reports; determining Site Categories; conducting laboratory inspections with NVSL; processing indemnity payment; meeting with industry on Program-related issues; and developing, supervising, coordinating and otherwise implementing the provisions of these Standards. The Program Veterinarian will, under conditions of confidentiality, have access to records of transfer permits and production information. USDA APHIS may utilize this information in epidemiological and economic investigations conducted by USDA staff or designees approved by the New England AVIC and for the generation of reports and information for utilization of the USDA APHIS ISA Technical Board in resolution of ISA incidents. The Program Veterinarian will be responsible for producing summaries of surveillance activities (as needed for significant changes in program or site status) for submission to: 1) the USDA APHIS VS New England AVIC or delegate; 2) the Maine DMR; 3) members of the ISA Technical Board; and 4) appropriate USDA APHIS VS Aquaculture staff.

To the extent feasible, the Program Veterinarian or Program staff will operate from an administrative base to be located in Maine and in the general vicinity of northeastern coastal salmon aquaculture operations. The Program Veterinarian or staff will also facilitate communications with contact personnel at Maine DMR, Maine Department of Inland Fish and Wildlife (IF&W), APHIS-approved

laboratories, NVSL staff, APHIS VS aquaculture staff, New Brunswick Department of Agriculture, Aquaculture and Fisheries (NBDAAF), Atlantic salmon producers, and other applicable parties (e.g. APHIS Legislative and Public Affairs personnel). Unless otherwise provided, all communications from the Program Veterinarian (and staff) will take place under conditions of confidentiality to the fullest extent of federal law. The Program Veterinarian (or designee) will serve as liaison between USDA APHIS VS and any other parties for all actions to be undertaken involving the detection, control, or elimination of ISAV- exposed, infected, or diseased fish under this program.

The Program Veterinarian will be a permanent member of the *ISA Technical Board*.

B. Access to sites

USDA APHIS VS personnel shall have access during normal hours of operations to all Atlantic salmon culture sites in order to carry out any and all aspects of these Program Standards.

C. ISA Technical Board

The ISA Technical Board membership is also defined in *Part I*. In short, there are 4 voting members, 4 proxies, and a non-voting chairman. Other non-voting members (i.e., subject matter experts or SMEs) may be appointed to the Board on an *ad hoc* or permanent basis by the APHIS VS New England AVIC or delegate at the suggestion of any member. Sub-committees may also be designated by the Board. A quorum for any issues requiring a vote will be all four voting representatives or their pre-selected proxies. The chairperson may only vote in the case of a tie and acts as a facilitator.

The purpose of the ISA Technical Board is to provide broad, balanced, and scientifically sound input to the New England AVIC, the Program Veterinarian, the Maine DMR, and/or the Maine Aquatic Animal Health Technical Committee (as defined in Chapter 24) . The Board provides recommendations based upon available information including laboratory testing results, epidemiological data, audit reports, or other information pertinent to reported disease risks or conditions requiring action under the terms of this program. The Board will also periodically review the ISAV Control Program (including these Standards) to recommend any revisions or other changes.

D. Confidentiality

Confidentiality will be maintained to protect proprietary information submitted by the participants in the program. USDA APHIS may release summary economic information pertaining to indemnification (if applicable), including total expenditures and the total number of producers receiving indemnity. Personal information including individual names and producer-specific indemnity amounts will not be released. Additionally, forms used to submit ISA surveillance or diagnostic samples to the laboratory are not required to contain a site identifier. The submitting accredited veterinarian will retain copies of the lab submission forms with the site identifiers; and will submit a copy to the Program Veterinarian (or designee).

Part III. Disease Surveillance and Investigation

A. Basic requirements

An ongoing surveillance program to facilitate early detection of both pathogen and disease is essential for the effective prevention, management, and control of a wide variety of aquatic diseases, including ISA. The following basic requirements are mandatory for producers participating in the ISAV Control Program. Participation in the ISAV Surveillance Program for all active marine sites as detailed in this document is mandatory for any producer to become and remain eligible for indemnification by USDA APHIS in the event that depopulation activities are required.

1. Any producer with marine Atlantic salmon culture sites in Maine will establish and maintain a veterinary-client-patient relationship with an accredited veterinarian. This veterinarian will have responsibility for implementing all applicable provisions of the Standards at participating sites under his/her supervision.

2. Accredited veterinarians (or trained delegates) will conduct surveillance on behalf of their clients and adhere to testing and reporting procedures for ISAV/ISA as described in these Standards.

3. Producers will develop and implement biosecurity protocols for use at marine sites, processing plants (if applicable), and vessels engaged in aquaculture operations throughout Maine (*See Appendix A*). These biosecurity protocols will be available to the Program Veterinarian (and staff). All marine net-pen sites and vessels will be subject to **biosecurity**

audits conducted by USDA staff or a third-party at least once a year, in addition to any internal audits. These audits will be completed by trained and experienced personnel as assigned by the Program Veterinarian. Records shall be kept of these audits, including recommendations for improvement and any corrective steps taken to address deficiencies.

a. Marine sites that are classified as ISAV Control Program Site Category 2-4 (See *Table 3*) may be subject to more frequent biosecurity audits.
b. If a processing plant is receiving fish from Category 2-5 sites, they may be subject to periodic biosecurity audits.

4. Producers shall make available a copy of their Integrated Pest Management Plan for the control of sea lice on salmonids when requested by the ISAV Program Veterinarian (or designee). Studies have indicated that *Lepeophtheirus* sea lice may be a vector capable of transmitting ISAV to Atlantic salmon (Nylund et al. 1994) or may reduce disease resistance (Valdes-Donoso, et al., 2013; Barker et al., 2019; Cai et al., 2022).

5. Accurate and timely **reporting** of all activities related to this program is essential. Reports and documents required include, but are not limited to, laboratory test reports; net-pen/site depopulation reports; cleaning and disinfection certificates; fish inventory documents; and permits.

a. All surveillance reports and actions regarding ISAV control measures must reference the site identification code. All surveillance documents, laboratory reports, and other documents as required will be forwarded to the Program Veterinarian (or designee). A flow chart reflecting the reporting mechanism is provided in Appendix D.
b. **Accredited veterinarians** shall, in accordance with Title 9 Code of Federal Regulations Part 161.4, report to the Program Veterinarian (or designee) and the Maine DMR any suspected or confirmed cases of ISAV within 24 hours of learning of these test results or diagnosis. Accredited veterinarians shall submit all diagnostic samples for ISAV to an APHIS-approved laboratory in accordance with instructions provided by these Standards, the Program Veterinarian (or designee), or the New England AVIC (or delegate), and shall follow all procedures as instructed by them. Accredited veterinarians shall use the *Sample Submission Chain-of-Custody form* for

regular and enhance surveillance and the USDA APHIS *Specimen Submission Form* (VS form 10-4) for samples sent to NVSL.

B. Surveillance Requirements

Minimum surveillance activities will involve onsite inspection by an accredited veterinarian, or technician under his/her supervision, as well as collection of appropriate samples (*Table 1*) for official testing. In addition, USDA APHIS personnel, under the direction of the Program Veterinarian (or designee) or the New England AVIC (or delegate), may collect samples as needed as part of this surveillance. The schedule of surveillance inspections depends upon each site's specified ISAV Control Program Site Category (*See Table 3* for Category Definitions). This schedule will be as follows, unless otherwise agreed by the Program Veterinarian (or designee), Aquaculture Health staff, and the ISAV Technical Board:

Monthly: Any sites meeting the criteria for ISAV Control Program Site Category 1. ***NOTE*:** New marine sites, Site Category 6 sites, or inactive marine sites returning to active status must initiate surveillance activities within 6 weeks of first introduction of salmonids to the site and monthly thereafter as long as they remain in Category 1.

Biweekly: Sites meeting the criteria for ISAV Control Program Site Category 2, as well as any other marine sites that are considered by the ISA Technical Board as potentially exposed to a Category 2-5 site (i.e., hydrographically linked).

Weekly: Sites meeting the criteria for ISAV Control Program Site Categories 3 thru 5.

As needed: the inspection schedule may be modified to resolve issues of ISA site status. Sampling frequency may also be adjusted at the discretion of the Program Veterinarian (or designee), in consultation with Aquaculture Health staff, NVSL, and the ISA Technical Board.

1. Surveillance inspections shall consist of visual inspections, by an accredited veterinarian or trained designee, of all net-pens on a site, review of weekly and/or most recent mortality figures, and collection of diagnostic samples for testing as described in these Standards. Inspection reports and other documents showing surveillance activities must be maintained and made available to the Program Veterinarian (or designee) upon request.

2. The accredited site veterinarian(s) will ensure monthly surveillance inspections of sites for which they have fish health responsibility. This should coincide with a scheduled dive to collect mortalities. More frequent surveillance requirements should include veterinary inspections as defined in III.B.1 above.

3. USDA-authorized personnel may also conduct sampling as necessary to comply with these Standards. This sampling option will be coordinated by the Program Veterinarian after consultation with the producer and accredited veterinarian.

4. When completion of harvest of all fish at a site is anticipated within the next month, a waiver of veterinary surveillance inspections may be obtained on a case-by-case basis from the Program Veterinarian (or designee).

C. Sample Collection

Active sites will be sampled as follows (refer to *Table 1*):

1. Appropriate numbers of moribund or recently dead fish will be collected per scheduled veterinary surveillance inspection. All fish deemed appropriate by the veterinarian will be collected at each routine monthly site visit, with a target of 5-10 fish. At the discretion of the site veterinarian, additional samples may be submitted.

2. Chain-of-custody documentation must accompany the submitted surveillance samples. A copy will be forwarded by the submitter to the Program Veterinarian (or designee).

3. Samples are submitted to APHIS-approved laboratories (*See Part III F*) for testing in accordance with diagnostic testing protocols in Part III D.

4. During every inspection, samples for RT-PCR and IFAT will be collected. Virus isolation samples will be collected for non-negative follow-up testing, Category 2 sites and higher, and at the discretion of the Program Veterinarian (or designee) or ISA Technical Board. IFAT impression smears will be archived at the receiving laboratory until needed or disposed of per NVSL protocols.

Table 1: Sample Types: to collect for testing under the ISAV Control Program.

TEST	Individual vs pool	Tissue	Collection type and timing
RT-PCR	Single fish	Duplicate - 0.25 cm^3 mid kidney	Duplicate - 2 ml tube/RNALater or other media acceptable to APHIS – in duplicate; collected during every inspection
IFAT	Single fish	2-3 Mid-kidney impressions	Frosted end slide; collected during every inspection
Virus Isolation (cell culture)	Individual or up to 5 fish per pool per net-pen	Duplicate - Kidney, spleen, heart	Duplicate -Specimen cup/approved media

D. Diagnostic Tests, Laboratory Standards, and Reporting

Table 2: Testing Workflow and Methods
For the official diagnosis by the APHIS-approved lab under the ISAV Control Program.

Workflow	Method	Assay	Comment
Primary	RT-PCR	Screening	Real-time RT-PCR; targeting segment 8
Confirmation of Primary	RT-PCR	Genotyping	Gel-based RT-PCR; targeting the putative HPR region of segment 6 (and additional segments as directed)
Confirmation of Primary	Partial Sequencing	Sanger	Targets putative HPR region of segment 6 (and additional segments as directed)
Secondary	Microscopy	IFAT	Samples collected at same time as the samples for screening, but not run until needed
Secondary	Virus isolation	Culture	Population resampled following non-negative primary test; also done as part of enhance surveillance
Secondary	Gross clinical signs	Observation	Submitting veterinarian collects and assesses

1. All official tests for ISAV will be performed by an APHIS-approved laboratory, except for gross clinical signs which will be evaluated by the accredited veterinarian or USDA-authorized sampler. Other APHIS approved methods may apply as they become available.

2. Approved laboratories must follow the most current NVSL ISAV testing SOPs. Primary screening assays should be initiated, completed, and reported within 10 days of sample receipt. The laboratory is responsible for maintaining records demonstrating competency. Records should be retained for at least 2 years. All remaining non-negative tissue samples and supernatants will be archived (for at least a 1 year) by the receiving laboratory after testing has been completed. Negative tissue samples and supernatants may be disposed of as set out in the NVSL protocols.
3. **Reporting requirements:**
    a. The approved laboratory shall report all results of surveillance sample testing to the Program Veterinarian (and staff), the Maine DMR, and the submitting accredited veterinarian within 10 days of sample receipt.
    b. Negative results of primary screening assays should be reported within 48 hours of test completion.
    c. Any non-negative result requires notification within 24 hours of the result to the Program Veterinarian (and staff), Maine DMR, the submitting accredited veterinarian, and the NVSL.
    d. Virus isolation results must be reported to the same entities as above within 30 days of sample receipt, 48 hours of test completion, or 24 hours of observation of ISAV CPE.
4. When reporting non-negative results to NVSL, the lab will coordinate shipping of appropriate samples for confirmation. This should occur within 24 hours of the primary non-negative result(s) and before confirmatory or secondary testing is initiated at the diagnostic lab.
    a. The diagnostic lab will include VS Form 10-4 with the samples and a copy sent by email to the Program Veterinarian, Staff, and NVSL (at *NVSL.Aquaculture@usda.gov*) with the shipment tracking information.
    b. If assigned, the FAD# and priority level will be noted on the Form 10-4 as communicated to the lab by the Program Veterinarian (or designee).
5. Confirmatory and secondary testing may proceed once samples have been prepared for shipping to NVSL.
6. For *Category 2b* status net-pens and sites, the remaining population should be resampled within 7 days of the initial non-negative results (refer to *Part IV A1*) for screening assay, with appropriate downstream testing, and virus isolation attempt. Virus isolation (cell culture)

requires 28 days barring issues; NVSL will complete partial genomic sequencing (i.e., segment 6 and/or 8) on all isolates and full genome sequencing when possible.

7. **Resolution of discrepant diagnostic results** will be determined by the ISA Technical Board. If needed they will consult with the applicable accredited veterinarian, the approved laboratory, NVSL, and/or Aquaculture Health Staff. If a site status remains unresolved after consultation, then issues surrounding discrepant or otherwise questionable results may be resolved through the following:
    a. Additional tests performed on archived samples by the approved laboratory and/or NVSL.
    b. Collection of additional samples from the site or net-pen to be tested by the approved laboratory and/or NVSL.
    c. Additional serial testing from the net-pen in question, additional net-pens, or other net-pen sites.

The Program Veterinarian (or designee), New England AVIC (or designee), and VS Aquaculture Health Staff shall consider recommendations made by the ISA Technical Board and will make final determinations (in conjunction with the Maine DMR) on all ISAV Control Program issues.

Part IV. Standards for Disease Control Actions and Management

A. ISAV Control Program Categories and Guidance

ISA Program Categories and their descriptions are found in **Table 3** below. The purpose of the classification system is to provide the Program Veterinarian, staff, and Technical Board guidance so that further evaluation, epidemiological investigation, and testing can be designed, ordered, and initiated as appropriate. These Categories are most often assigned per net-pen.

Table 3: ISAV Control Program Categories and Guidance

Category	Category Description	Guidance
Category 1 (Presumed negative)	ISAV has NOT been detected in regular or enhanced surveillance testing in a net-pen or site over the last two months or longer.	Monthly surveillance inspections.
Category 2a (Non-negative - low concern)	Any ISAV detection result in one or more fish is considered suspect and warrants further evaluation. **OR** primary screening was unconfirmed. **OR** ISAV HPR0 has been detected and confirmed.	Continue monthly surveillance. *Follow-up sampling* **may** occur. **May** consider increasing surveillance to biweekly. *Recategorize* if Category 1 criteria are met.
Category 2b (Detection – elevated concern)	ISAV HPR-deleted has been detected in one fish by one or more *independent tests* in a single net-pen.	*Follow-up sampling* occurs. Biweekly surveillance. *Quarantine* **may** be ordered. *Recategorize* cage if Category 1 criteria are met.
Category 3 (Asymptomatic infection)	ISAV HPR-deleted has been detected and confirmed by 2 *independent tests* in at least two fish in a single net-pen; **OR** testing has resulted in HPR-deleted ISAV detected in multiple fish by only one test	Increase surveillance to weekly. *Quarantine* **may** be ordered. *Depopulation* of net-pen **may** be ordered by *Maine DMR*. *Recategorize* cage if Category 1 criteria are met.
Category 4 (Diseased)	As for Category 3 above, plus increased morbidity consistent with clinical ISA (as diagnosed by an accredited veterinarian) and/or increased mortality	*Quarantine* ordered. *Depopulation* of net-pen **may** be ordered by *Maine DMR*. Post-depopulation, recategorize site as appropriate.
Category 5 Fallow	Net-pen or site previously classified as Category 2 through 4 has been fallowed.	Cleaning and Disinfection of all equipment

Independent tests to be considered in the determination of a "confirmed net-pen" 1) gross clinical signs, 2) RT-PCR, 3) IFAT, 4) and virus isolation; also, for consideration in determining Category Status - sequence of segment 6 (HPR) and any additional segment sequences.

1. **Follow-up sampling**:

   For HPR0 non-negatives, follow-up sampling is not required, but may be done at the discretion of the APHIS-accredited veterinarian in consultation with the Program Veterinarian. For HPR-deleted, follow-up sampling should occur within 7 days of the non-negative report. Samples should be collected from the suspect net-pen whenever possible; if an adequate number of moribund fish are not available from that net-pen, samples may be collected from other net-pens on the site. The required follow-up samples will be sent to both the diagnostic lab and NVSL - samples for RT-PCR and viral culture will be sent to both; and samples for IFAT will be sent to the diagnostic lab only.

2. **Recategorization of a net-pen/site**:

   Net-pens and sites will be categorized or re-categorized as often as necessary to reflect testing results and management actions taken under this section, or taken at the discretion of individual producer policy, if that policy does not conflict with the intent of these Standards. The site will be designated with the highest net-pen Category number it contains. For example, if it contains both a Category 2 and 3 net-pen, it will be designated as Category 3 until that net-pen is removed. If a site contains a single Category 3 or higher net-pen, once the fish are removed from that net-pen, the site will revert to Category 2, and undergo biweekly testing until there have been 2 months of negative testing results.

   Category 2a net-pens/sites will revert to Category 1 after at least two consecutive (i.e., two months) negative monthly veterinary surveillance inspections. Category 2b and 3 net-pens/sites will revert to Category 1 once criteria is met. Once a diseased net-pen is removed, Category 4, the affected site will be categorized as Category 2b until the criteria for Category 1 are met. Recategorization may also occur at the discretion of the ISA Technical Board.

3. **Quarantine:**

   Quarantines will be issued by the regulatory authority of the Commissioner of the Maine DMR. Quarantines will be released only after fish have been moved in compliance with program standards and all nets, pen equipment, and other fomites have been properly cleaned and disinfected, or replaced. Fish or population cohorts considered exposed to ISAV as a result of interactive epidemiological factors may be considered for quarantine depending on the particular circumstances pertaining to each outbreak. Conditions of quarantine include: 1) the implementation of a biosecurity plan is required to be approved by the Maine DMR; 2) visitation is limited by the biosecurity plan; 3) disposition of all quarantined fish must be done in a manner

approved by the Program Veterinarian (or designee) and the Maine DMR; and 4) no equipment or vessels are to move between the quarantined site and other sites unless authorized by the Program Veterinarian (or designee) and Maine DMR.

4. **Depopulation**:
   a. The Program Veterinarian (or designee) will serve as liaison between USDA APHIS VS and all other parties for all actions to be undertaken involving the depopulation of any stock.
   b. When net-pens are declared infected or diseased by the Program Veterinarian (or designee), in consultation with the ISA Technical Board and USDA APHIS VS Aquaculture Health Staff, depopulation orders will arise from the regulatory authority of the Commissioner of the Maine DMR per their *Chapter 24.21* regulations.
   c. The producer will develop a site-specific action plan for the control and management of the disease, in consultation with the Program Veterinarian (or designee) and ISA Technical Board. The Program Veterinarian (or designee) may request inventory, mortality data, etc. from the affected site.
   d. Any depopulation ordered by the Maine DMR must be complete within the time frame set by the order. If unusual circumstances such as large-scale depopulation or weather-related factors intervene, a request for an extension of the depopulation completion timeline must be submitted in writing to the Commissioner of the Maine DMR before the original deadline has passed. Depopulation must be accomplished minimizing exposure of all other fish at the site or in adjoining waters, and in accordance with *Appendix A* of this document. Standards for fish harvesting, transportation, and processing are also included in *Appendix A*. Depopulated fish may be moved to composting sites, landfills, or fish processing plants only after meeting all applicable federal and state regulatory criteria. All methods of harvest or transport for disposal shall comply with either the stipulations of these Standards, Maine *MRSA §6071* and DMR Rules *Chapter 24.21,* and/or any pertinent DMR Emergency regulations. Category 3, 4, or 5 fish (infected and diseased) that are harvested by live haul will be transported to processing plants in a manner that does not allow untreated contained water to be exchanged with or otherwise contact the environment during transport. The Program Veterinarian or other APHIS personnel appointed by the New England AVIC will oversee the depopulation procedures for as many units as may be practical.
   e. Further actions to be taken: 1) the Program Veterinarian (or designee) will notify the New England AVIC (or delegate); 2) the ISA Technical Board may be convened; 3) additional

epidemiological information may be requested from the producer; and/or 4) consultation with USDA APHIS VS NVSL or VS Aquaculture Health Staff may occur.

B. Indemnity

As stipulated in Title 9 CFR Part 53.10e, , indemnity payments will be made to producers complying with the Standards, for fish destroyed due to ISA per federal mandate. These may include ISAV-infected as well as ISAV-exposed fish. The Program Veterinarian (or designee) and New England AVIC will work with the producer and state to process indemnity payments through APHIS Aquaculture staff.

Upon determination that fish are to be depopulated an indemnity estimate worksheet will be prepared and signed by the producer or producer's representative and the Program Veterinarian or New England AVIC.

In the case of non-marketable fish, indemnity will be paid on a per-fish basis using stocking inventory documents, mortality figures, and other information available. Indemnity for marketable fish may be paid on a per-fish basis using an indemnity scale with the USDA paying any difference between individual fish salvage proceeds and the per-fish indemnity value.

The USDA APHIS VS Appraisal and Indemnity Claim (VS form 1-23) is the official document to process all indemnity claims for this program. Indemnity payments will be executed only after the site is properly cleaned and disinfected in accordance with *Part IV D* and *Appendix A* of these Standards.

C. Permitting

All fish as well as nets, equipment, and other fomites capable of transmitting ISAV must be permitted for removal from all sites with a Category status of 3, 4, or 5. Permitting will be coordinated between USDA APHIS VS and the Maine DMR to allow movement of confirmed positive fish to processing plants, composting facilities, rendering, or landfill sites. A VS form 1-27, Restricted Movement Permit, will be required. For international movement, the importing country's permitting requirements must also be met.

D. Cleaning and Disinfection

If any net-pen or site depopulation is ordered as a result of ISA surveillance, cleaning and disinfection must occur after any complete removal of fish from a site. The level, specification, and schedule for cleaning and disinfection after any depopulation is ordered for any net-pen or site will follow established guidelines (*See Appendix A*). The ISA Technical Board may be convened to review issues surrounding cleaning and disinfection and make recommendations to the Program Veterinarian.

Site-specific cleaning and disinfection programs shall be developed and implemented at all finfish culture sites, and must include at least the following elements:

- All site personnel and management shall be trained in proper disinfection procedures.
- Documentation shall be maintained in order to verify consistent implementation and identify employees responsible for their implementation.
- Assure that contractors or other visitors understand and follow disinfection guidelines and all other relevant protocols.

E. Fallowing

Fallowing must occur following removal of any population of fish, whether infected or not. The minimum fallow time is 30 days. In the absence of disease, fallowing time begins upon fish removal. In the case of disease-causing ISAV, fallowing begins only upon completion of all cleaning and disinfection procedures of nets, pens, equipment, and other fomites as required. Fallows are to be synchronized with neighboring sites in restricted Fish Health Zones. The Maine DMR has final determination of fallow duration.

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APPENDIX A: Fish Health, Biosecurity, and Cleaning & Disinfection

These guidelines are intended to reduce the risk of the introduction and spread of infectious diseases (including but not limited to ISA) at Atlantic salmon production sites. Each producer shall develop company and site-specific protocols addressing at a minimum the types of activities that can impact fish health or biosecurity at production sites,

Effective Disinfectants

The effectiveness of most disinfectants is greatly reduced by organic material. All objects must be thoroughly cleaned to remove organic materials prior to disinfecting.

The following is a list of disinfectants that are effective against ISAV:

- sodium hypochlorite (100mg/l free chlorine for minimum of 10 minutes)
- iodophor (100ppm for 10 minutes or 250ppm for a few seconds)
- formaldehyde (1.0% for 16 hours)
- formic acid (pH <4 for 24 hours)
- sodium hydroxide (pH > 12 for 7 hours)
- heat (>55C for > 5 minutes)
- ozone (8 mg/l/min for three minutes – corresponding to a Redox potential of 600-750mV)
- UV radiation (120mJ/cm2)
- Virkon® S (2% solution/10 minutes; followed by water rinse)
- Sodium thiosulfate can be used to neutralize chlorine or iodine-based disinfectants.

Note: The choice of a particular disinfectant should be based on efficacy in a particular application

Cleaning & Disinfection Levels

Three levels of vessel and equipment disinfection are to be used. The minimum level of disinfection required is determined by the operational circumstances as defined in the Table below.

OPERATIONAL CIRCUMSTANCES	DISINFECTION LEVEL
Travel from any Category 1 site/zone to any Category 1-5 site/zone	1
Travel from any Category 2 site/zone to any Category 1 site/zone	2
Travel from Category 2 site/zone to any other Category 2-5 site/zone	1
Travel from Category 3-5 site/zone to any Category 1 or 2 site/zone	2 or 3*
Travel from Category 3-5 site/zone to any other Category 3-5 site/zone	1 or 2*
Travel from a wharf that services vessels coming from Category 2-5 to a Category 1 site or zone	2 or 3*

** As determined by DMR and ISA Program Veterinarian (or designee)*

Level 1 Cleaning and Disinfection:

- Prior to use, ensure disinfectants are approved for discharge under the producer's Maine DEP permit.
- Establish a "clear deck".
- Any ropes, straps or equipment removed during the process of establishing a "clear deck" should be cleaned and disinfected prior to stowing.
- Thoroughly clean all surfaces from the water line up of any organic material or inorganic particulate matter.
- Special efforts should be made to remove any fats or oils.
- Coat and scrub all surfaces using an approved disinfectant allow appropriate contact time.
- A low-pressure applicator may be used to apply disinfectants.
- A high-pressure washer may be used for cleaning and a steam pressure washer may be used for both cleaning and disinfection.
- Fill out and sign cleaning and disinfection log.

Level 2 Cleaning and Disinfection:

Perform all Level 1 cleaning and disinfection protocols. In addition, perform the following Level 2 protocols:

- Internally inspect, cleanse, and disinfect any fish pumps and vessel wells.
- Ensure that disinfectants are repeatedly cycled through all pumps, pipes, hoses and/or valves that may have contacted fish, fish water or blood-water.

Level 3 Cleaning and Disinfection:

- Perform all Level 2 cleaning and disinfection protocols. In addition, slip or careen the vessel; clean, scrape, wash, and disinfect the hull.
- If it is not possible to careen the vessel, producers should discuss alternatives with the DMR and ISA Program Veterinarian (or designee).

Cleaning and Disinfection Protocols for Specific Areas of Operation:

A. Vessels & Wharves

- Vessel traffic between marine sites and between wharfs should be minimized.
- Vessel traffic between fish health zones should be minimized.
- All vessels must maintain a disinfection log that documents the cleaning and disinfection procedures used on the vessel.
- Disinfection logs for site-specific skiffs can be maintained on the farm site rather than carried on-board.
- At a minimum, the disinfection log must identify what specific areas of the vessel were cleaned and disinfected; the cleaning, and disinfectant agents used, the date of such procedures, and the signature of the responsible employee or vessel captain.
    - All vessels shall have their hulls cleaned and scraped regularly, at least annually, to minimize biofouling.
    - All vessels carrying live or dead fish must clean and disinfect all areas of the vessel from the waterline up before and after each trip.
    - Particular attention should be paid to areas of the vessel and fish handling equipment that come in direct contact with fish or water fish have been in.

    - All vessels carrying live or dead fish must fill out their disinfection log after each day they have carried fish.
    - When cleaning and disinfecting any vessel, particular attention should be paid to areas that are difficult to access. Wherever possible, vessel and equipment design should minimize such areas.
    - Vessels operating in fish health zones with ISAV Control Program Category 2-6 sites must have their hulls cleaned and scraped twice annually or once annually and use an effective anti-foulant hull paint.
- All vessels are subject to third party biosecurity audits per DMR *Chapter 24* regulations.
- At the wharves used to access sites, boom truck operators and loading crews must avoid all spillage. If spillage does occur, every effort should be made to contain the spillage and clean and disinfect the area.
- Disinfect vessel and all gear after leaving the wharf.
- Disinfect boom truck and all gear including straps after loading/unloading the vessel.

B. Personnel & General Equipment

- Limit traffic to sites and require that everyone going to the site properly disinfects on the wharf or vessel, and that everyone leaving the site properly disinfects on the vessel.
- All sites must maintain a visitor log.
- All people traveling to a site must wear footgear that can be disinfected by stepping into a footbath.
- Footbaths must be present, and properly maintained, throughout the site and on vessels.
- Everyone who comes in contact with dead fish, moribund fish, processed fish or fish parts, and blood-water shall properly clean and disinfect their gear and themselves before leaving the site/vessel.
- Keep employees and their gear, site-specific when possible. Proper disinfection and air-drying of all gear must be enforced between sites when this is not possible. Properly clean and disinfect all equipment after each use.
- Do not use wooden equipment, vessels or barges - they cannot be properly disinfected. Wooden pallets are permissible for one time use only, such as feed delivery.
- Minimize sharing of equipment between sites.

C. Containment Nets & Pens

- When replacing nets, they should be cleaned on land when possible.
- Nets and pens must be cleaned of all organic material before disinfecting.
- All nets from Category 2 through 5 sites will be taken to land for cleaning and disinfection.
- Nets from Category 2 through 5 sites being transported to shore will be contained in a manner which prevents the loss or spillage of organic matter.
- Care should be taken to ensure that the cleaning/disinfecting procedure used for nets does not adversely affect the breaking strength or anti-foulant treatment (if applicable).

D. Divers and diving gear

- Properly disinfect diving gear before first net-pen, between net-pens, and after last net-pen.
- Dive the youngest fish first when diving multiple sites in a day.
- Dive net-pens with elevated mortality last; if there are suspect and/or positive net-pens, dive these last and disinfect between diving on net-pens by full immersion.
- All diver equipment should be site-specific, when possible. If a diver must dive more than one site using the same gear, it is imperative that all gear is disinfected between sites. Ideally, gear should be disinfected after the last net-pen at the first site and allowed to air dry. If this is not practical, gear

should have at least a 10-minute contact time with iodophor solution before being used at the second site.

- Diver attendants shall wear designated site-specific and mort-specific rain gear. This gear must be properly cleaned and disinfected after each use. Boots shall be properly disinfected after each mort dive.
- Disinfect net-pen handrails and net areas above the water line that mort bags and divers come in contact with.
- If possible, a separate site-specific vessel (steel or fiberglass construction) should be dedicated to diving only.
- During dives, when possible, separate mort bags should be used for each net-pen; otherwise, bags should be alternated between net-pens so that one bag will soak in disinfectant for 10 minutes or more.

E. Dead fish & Blood-water collection, storage, and disposal

- Dead fish, moribund fish or blood-water shall not be released into the marine environment.
- All attempts should be made to prevent leakage and spills during harvest and transport.
- Collect all dead fish at least once weekly, weather permitting. More frequent collection should occur if mort numbers are elevated.
- If the mortality level meets or exceeds 0.05% per day, mortalities are to be removed daily from net-pens or sites. At this level, each pen must have its own mort bag and any weak fish should be dipped from the surface.
- Use only mort containers in good condition, never cracked and leaky ones.
- Use plastic liners in mort containers. Cover mort containers with properly fitting lids.
- All mortality-related equipment should be kept separate and away from other equipment.
- All mortalities are to be taken ashore and disposed of at a Maine Department of Environmental Protection (DEP) approved facility.
- Use mort-specific equipment for storage and transport of dead fish. Mark containers “morts only”.
- If possible, mort containers should be site specific.
- Mortalities should be removed from the site after each dive, or as soon as possible.
- Do not store mortalities on site, if possible. If morts are stored on site at all, store mort containers away from feed. Place a footbath with disinfectant in the immediate vicinity of the mort containers.
- Disinfect area beneath and surrounding mort containers whenever it is removed for disposal of dead fish. Promptly clean and disinfect any spillage from mort containers.
- Clean and sanitize mort containers before returning to a site. This is best done immediately after disposal.

F. Broodstock & Eggs

Although the best current scientific information indicates the risk of vertical transmission for ISA is low, the following guidelines are recommended as good husbandry practices designed to reduce the potential risk of vertical transmission in general.

- Lethal sampling and disease testing should be conducted on all broodstock.
- No gametes should be used from clinically infected broodstock sites.
- No gametes should be used from individual broodstock that test confirmed positive for any pathogen of regulatory concern (including non-pathogenic genotypes). Refer to DMR *Chapter 24* regulations.
- Eggs and juvenile stages at freshwater facilities should not share the same facility area or water mass with broodstock moved from marine sites.
- Any movement of broodstock or eggs must be permitted under DMR *Chapter 24* regulations.

APPENDIX B: ISAV Control Program Contacts

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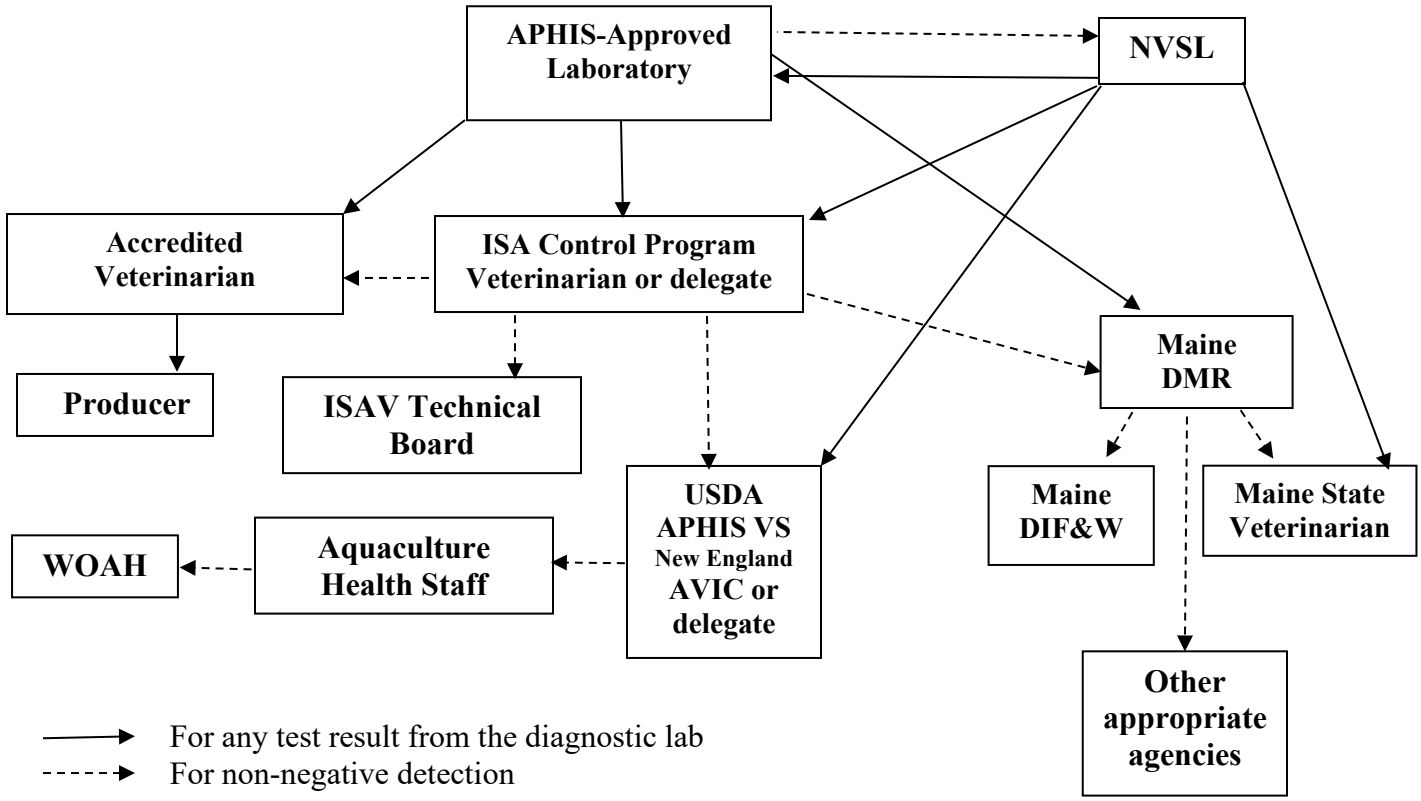
APPENDIX C: USDA APHIS ISAV Control Program Flow Chart

Test Results Notification Flow Chart



APPENDIX D: USDA Sample Submission Form

USDA APHIS ISA Control Program

ISAV Sample Submission Chain-of-Custody Form

On the top copy, list the pen or cage designation number(s), the year class of fish sampled, and the number of fish sampled per cage, whether signs of ISA are present, and the date samples were obtained and submitted. On the middle and bottom copies, please also list the marine site by name and Maine DMR code identifier. The collecting inspector should sign and date the form and indicate how samples were sent to the laboratory. All information will be treated with confidentiality to the fullest extent of federal law.

USDA ISA Control Program Accession #:

Laboratory Accession #: _____

Tag # (opt.)	Fish #	Cage #	Viro Pool	YC	Date sampled	Water Temp °C	Sea lice counts	Date submitted	Lab Use Only		
									PCR	IFAT	Culture
	1										
	2										
	3										
	4										
	5										
	6										
	7										
	8										
	9										
	10										

Clinical Disease Present? _____

Gross Pathology (List signs by fish #) _____

_____

_____

_____

_____

_____

Site/Cage Mortality (Low, steady, increased) _____

Samples collected by: _____

Samples processed by: _____

Samples submitted via:

[ ] Diagnostic Laboratory courier [ ] US Mail (Priority Overnight Express)

[ ] ISA Control Program Manager [ ] FedEx

Send report to (print name): _____, DVM

Supervising USDA APHIS VS Accredited Veterinarian _____

ISA Control Program Manager Countersignature _____

Date_____

Maine DMR Site Identifier Code_____

White Copy: Laboratory Yellow Copy: USDA Pink Copy: Submitting Veterinarian

APPENDIX E: Current revision (2023) input - ISAV Technical Board members & proxies and Subject Matter Experts (SMEs)

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- To navigate to specific bookmarks, click on Bookmark, choose the appropriate bookmark and then 'Go To'.
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