

Maine Infectious Salmon Anemia Virus Control Program Standards

USDA APHIS Veterinary Services

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Table of Contents

EXECUTIVE SUMMARY	3
INTRODUCTION AND BACKGROUND	4
MAJOR ISAV CONTROL PROGRAM COMPONENTS	9
PART I. DEFINITIONS	11
PART II. ADMINISTRATIVE PROCEDURES	16
A. SUPERVISION OF THE ISAV CONTROL PROGRAM.....	16
B. ACCESS TO SITES	17
C. ISA TECHNICAL BOARD.....	17
D. CONFIDENTIALITY	18
PART III. DISEASE SURVEILLANCE AND INVESTIGATION.....	18
A. BASIC REQUIREMENTS	18
B. SURVEILLANCE REQUIREMENTS	19
C. SAMPLE SPECIFICATIONS.....	20
D. DIAGNOSTIC TESTS AND DIAGNOSTIC TESTING STANDARDS	21
E. BIOSECURITY AUDITS.....	22
F. REPORTING.....	23
PART IV. STANDARDS FOR DISEASE CONTROL ACTIONS AND MANAGEMENT	24
A. ISAV CONTROL PROGRAM CATEGORIES.....	24
B. QUARANTINES	26
C. INDEMNITY	26
D. DEPOPULATION	27
E. PERMITTING	28
F. CLEANING AND DISINFECTION.....	28
G. FOLLOWING.....	28
APPENDIX A: FISH HEALTH, BIOSECURITY, AND CLEANING & DISINFECTION	35
EFFECTIVE DISINFECTANTS	35
CLEANING & DISINFECTION LEVELS.....	35
CLEANING AND DISINFECTION PROTOCOLS FOR SPECIFIC AREAS OF OPERATION:	36
A. VESSELS & WHARVES	36
B. PERSONNEL & GENERAL EQUIPMENT	37
C. CONTAINMENT NETS & PENS	37
D. DIVERS AND DIVING GEAR.....	37
E. DEAD FISH & BLOOD-WATER COLLECTION, STORAGE, AND DISPOSAL.....	38
F. BROODSTOCK & EGGS	38
APPENDIX B: NOTIFICATION GUIDANCE FOR LABORATORY AND VS PERSONNEL...39	
APPENDIX C: ISAV CONTROL PROGRAM CONTACTS	41
APPENDIX D: USDA APHIS ISAV CONTROL PROGRAM FLOW CHARTS.....	44
APPENDIX E: USDA SAMPLE SUBMISSION FORM.....	45
LIST OF TABLES	
TABLE 1: SAMPLE SPECIFICATIONS FOR ISAV CONTROL PROGRAM APPROVED ASSAYS.....	21
TABLE 2: ISAV CONTROL PROGRAM CATEGORIES AND DESCRIPTION.....	24

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*). 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, in part, on compliance with these Standards, and upon availability of any indemnity funds (*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. These Standards have been revised twice since then, by the [ISA Technical Board](#), to reflect updated information regarding ISAV/ISA transmission, diagnostic tests, and control processes.

Introduction and Background

Infectious salmon anemia (ISA) is an important 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 Organization for Animal Health (OIE) by competent authorities worldwide. All suspect detections of ISAV or outbreaks of ISA in the U.S. are reportable to USDA APHIS Veterinary Services as the Federal competent authority for animal health.

ISAV is found in farmed or free-ranging fish in freshwater and marine settings. While ISA is a disease of marine farmed Atlantic salmon (OIE, 2016), the species in which ISAV detections have been made include Atlantic salmon (*Salmo salar*), 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 (>56°C), extreme pH (<4 or >12), UV, ozone and a variety of disinfectants containing chlorine, iodine or potassium peroxydisulfate 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 (OIE, 2016).

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 (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 affected fish (OIE 2016).

Morbidity and mortality due to ISA are highly variable. Cumulative mortality varies greatly from near zero to a majority of fish on a given site. Outwardly, affected fish may show few signs of infection or disease until late in a given disease cycle, or may alternatively exhibit a variety of associated signs including pale gills, exophthalmia or bulging eyes (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 or swelling of the liver, spleen and kidney capsule. Microscopic lesions can include congestion and necrosis in the liver, heart, blood vessels, spleen and kidneys. (OIE, 2016).

Epidemiological factors

Transmission of ISAV is believed to occur principally through horizontal routes. Virus is shed into the environment through blood, mucus, feces, urine, skin or carcasses of infected fish (Vike et al., 2014). Epidemiological investigations suggest the virus also spreads between aquaculture sites via untreated wastes and water from harvest operations or 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 this theory is debated, recent evidence suggests at least some ISAV genotypes may be transmitted vertically (i.e., from parent to offspring) through infected eggs (Marshall et al., 2014).

Arthropods such as sea lice (*Lepeophtheirus salmonis* and *Caligus* sp.) are considered potential pathogen vectors, but their significance in ISAV transfer is still under study (Valdes-Donoso et al.,

2013). Secondary effects such as stress or tissue damage may also increase disease susceptibility (Rolland and Nylund, 1998a). Little supporting evidence is available to demonstrate that other marine species act as efficient ISA virus carriers or reservoirs capable of infecting cultured Atlantic salmon.

Genomic information

Sequence comparisons of genome segments 2, 5, 6 and 8 of ISAV isolates identify two distinct genotypes: Genotype I and Genotype II (Kibenge et al., 2001; Krossoy et al., 2001; Kibenge et al., 2009). Genotype I, also termed ‘European’, is commonly affiliated with Atlantic salmon in Norway, Scotland, Shetland Islands and the Faroe Islands; however, there have been several detections of this genotype in North America (pers. comm. Michael Beattie, NBDAAF). Genotype II, also termed ‘North American’, is predominantly associated with Atlantic salmon in North America. Studies of isolates from Norway, Scotland, and Canada indicate that these two subtypes appear to have diverged more than 100 years ago (Krossoy et al., 2001). A 2007 outbreak in Chile involved Genotype I and has been tentatively linked to trade of eyed embryos from Norway (Kibenge et al., 2009; Vike et al., 2009).

Phylogenetic studies of surface glycoprotein gene sequences encoded on ISAV segments 5 and 6 further subdivide Genotype I into three geno-groups, EU-G1, EU-G2 and EU-G3 (Nylund et al., 2003; Devold et al., 2006; Kibenge et al., 2009). Genotype II variability appears less substantive, so this genotype has not been similarly subdivided. Genotyping and geno-grouping (if an EU genotype) are important for phylogenetic tracing.

Additionally, genetic variation among isolates has been investigated for use as markers of virus virulence or dissemination 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’ (OIE 2016). In contrast, HPR variants having full-length HPR sequences are termed ‘HPR0’. To date HPR0 variants have 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 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) are occasionally reported from single fish or sites. 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.

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). The majority of pathogenic ISAV isolates confirmed to date in Maine and New Brunswick have been classified Genotype II (Nylund, et al., 2003; Kennebec River Biosciences, Inc. pers. comm; New Brunswick Department of Agriculture, Aquaculture & Fisheries, pers. comm.). In contrast, the HPR0 variants detected in Maine and Maritime Canada are typically classified Genotype I (Ritchie et al., 2001; Cook-Versloot et al. 2004; Gustafson et al., 2008).

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 affected regions. 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 resolution of ISA disease in the region (Ellis et al., 2006). The last detection of ISAV (HPR-deleted) associated with this event occurred in February 2006. Several findings have been reported in Canada since then, though these have been localized and responsive to control. However, ISAV HPR0, as confirmed by sequence analysis, has been periodically detected by RT-PCR at sites in Maine and Maritime Canada since 2003.

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 al., 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, 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 joint collaboration among 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, APHIS-approved diagnostic laboratories, 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 (subject to availability of funding). Other related aspects include, the development of action plans, risk identification and mitigation, movement restrictions, selective depopulation, synchronized fallowing, overall management coordination and shared communications among marine farming operations involving Atlantic salmon.

Frequent, targeted surveillance ensures that ISAV incursions will be quickly detected when present. Testing with currently approved 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 HPR-deleted ISAV eliminates a continuing transmission risk. Finally, indemnity funds, when available, provide financial relief to producers while encouraging prompt reporting and compliance with the Standards.

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 farmed salmon producers in Maine and Maritime Canada utilize highly qualified personnel experienced in all aspects of fish culture, husbandry, and health management. Producers have established comprehensive internal procedures for increased disease surveillance, and utilize working

relationships with aquaculture veterinarians and diagnostic laboratories to provide further technical expertise. The producers comply with applicable fish health inspections needed to meet import and export regulatory requirements for fish movements. Current state-level requirements in Maine include, at minimum, annual health inspections for all lots of Atlantic salmon (or other salmonid fish species) before transfer from freshwater hatcheries to marine sites; and testing of Atlantic salmon broodstock at spawning. 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 objectives 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 and reporting, and restrict finfish aquaculture vessel and equipment movements. In addition, these Standards were developed to specify the testing procedures 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, and thorough egg disinfection protocols at fertilization and water hardening. Due to shared water resources, as well as potentially shared personnel and equipment by producers 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 considered essential.

Part I. Definitions

Accredited veterinarian: a veterinarian holding a current Maine State veterinary license, whom 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 II F](#)

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

Active site: a marine finfish culture site as designated by the Maine DMR, and at which fish are present

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) [See Part III F](#)

Assays: specific diagnostic tests for the detection of pathogens

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 II E](#))

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 eliminating a disease outbreak

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.

Disease of Regulatory Concern: infectious disease that has been demonstrated to cause a significant increase in the risk of mortality among salmonid populations in the State of Maine. Diseases 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 G](#)*

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 G](#)*

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

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: Sequence analysis reveals a putative “full-length” nucleotide sequence (105 nucleotides = 35 amino acids) for the highly polymorphic region of gene segment 6 which encodes the stem of the HE protein of ISAV.

HPR-deleted ISAV: Sequence analysis reveals gaps in the nucleotide sequence for the highly polymorphic regions of gene segment 6 which encodes for a shortened stem region (11 to 34 amino acids) of the HE protein of ISAV.

Indemnity: compensation paid to producers of Atlantic salmon or other cultured susceptible species in exchange for depopulation; *See [Part IV C](#)*

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](#)*

Integrated Pest Management Plan: Producer's IPM program considered the State of Maine Program when submitted by the producer to enroll in the Maine ISA Program.

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

ISA Program Categories: staged categories of ISA status; *See [Table 2](#)*

ISAV Control Program Veterinarian: USDA APHIS SPRS VS employee, assigned by the VS District 1 Director (DD) or District 1 Assistant Director (AD), to manage the ISA Program in Maine, and who reports to the District 1 DD or AD.

ISA Technical Board: a group of four voting members approved by the District 1 AD 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](#)*

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 2](#))

Net-pens: also called “net-pens” or “pens”; plastic or steel structures of differing sizes and shapes designed to contain variable numbers of fish

Non-pathogenic: not known to induce 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

Permit: a USDA APHIS Veterinary Services document entitled “Permit for Movement of Restricted Animals” (VS form 1-27) which allows for the bio-secure movement of fish from sites; may also refer to the vessel or fish transfer permit required by the Maine DMR

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 B](#)

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, a process where RNA is converted to complimentary DNA copy which is amplified in a cyclic fashion using a sequence-specific primer that binds to the gene target of a particular organism

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.

Sequence: (*Noun*) The order of nucleotide bases in a DNA or RNA molecule; the order of amino acids in a protein molecule; the order of amino acids translated from a DNA or RNA molecule.

(*Verb*) To determine the genetic code (nucleotides or amino acid translation) of a specific portion of the ISAV genome

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

Site: a specific area or facility where fish are raised

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

Site veterinarian: a licensed and [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

Virus Isolation: growth of a virus in a particular cell line; See [Cell culture](#)

Year class: grouping of a population of fish of the same age/hatch year; and/or grouping by year of transfer to a marine site

Part II. Administrative procedures

A. Supervision of the ISAV Control Program

The USDA APHIS VS District 1 Office for Surveillance, Prevention and Response Services (SPRS), located in the New England Area office in Sutton, MA, has administrative and supervisory responsibility for administering and managing all USDA, APHIS, VS, SPRS sector programs in New England, including the ISAV Control Program in Maine. The District 1 Director (DD) (or Assistant Director (AD), as delegated) will appoint a VS employee to oversee the ISAV Control Program, designated as the Program Veterinarian.

ISAV Control Program Veterinarian: The Program Veterinarian is responsible for overall field management of the ISAV Control Program in Maine and reports directly to the District 1 AD. 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; reviewing laboratory procedures; processing indemnity payment; and meeting with industry on Program-related issues. 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 District 1 AD 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 District 1 AD (or delegate), 2) the Maine DMR, and 3) members of the USDA APHIS ISA Technical Board.

The Program Veterinarian is responsible for management and administration of the ISAV Control Program. Major duties and responsibilities include coordinating and implementing all aspects of the ISAV Control Program (including these Standards) in Maine, and submitting reports to the District 1 AD. 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 will also facilitate communications with contact personnel at Maine DMR, Maine Department of Inland Fish and Wildlife (IF&W) , approved

laboratories, NVSL staff, APHIS VS aquaculture staff, New Brunswick Department of Agriculture, Aquaculture and Fisheries (NBDAAF) or other applicable parties (e.g. APHIS Legislative and Public Affairs personnel). Unless otherwise provided, all communications from the Program Veterinarian (or staff) will take place under conditions of confidentiality to the fullest extent of federal law. The Program Veterinarian will develop and coordinate the dissemination of all necessary USDA APHIS VS forms specific to the ISAV Control Program, and develop, supervise, coordinate and otherwise implement the provisions of these Standards. The Program Veterinarian 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 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 may be appointed to the Board on an *ad hoc* or permanent basis by the APHIS SPRS District 1 AD 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 District 1 DD or AD, the Program Veterinarian, the Maine DMR and/or the Maine Aquatic Animal Health Technical Committee. The Board provides recommendations regarding 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.

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 1) depopulation activities occur; and 2) funds become available.

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 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.

4. [Biosecurity audits](#) of active sites and vessels shall be conducted at the frequency required by the Maine DMR [Chapter 24.21](#) regulations. These audits will be completed by trained and experienced personnel as assigned by the Program Veterinarian.
5. Producers must submit an [Integrated Pest Management Plan](#) for the control of sea lice on salmonids at the time of enrollment into the ISA Program. Studies have indicated that *Lepeophtheirus* sea lice may be a vector capable of transmitting ISAV to Atlantic salmon (Nylund et al. 1994).

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 for ISAV assays. In addition, USDA APHIS personnel under the direction of the Program Veterinarian or the District 1 AD (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 2](#) for Category Definitions). This schedule will be as follows:

Monthly: Any sites meeting the criteria for ISAV Control Program Site 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. Note: if a detection of an HPR-deleted genotype of ISAV has been made in the lower end of New Brunswick's ABMA1 (around Deer Island or Campobello Island), sampling of all active marine sites in Cobscook and Passamaquoddy Bays may, at the discretion of the ISA Technical Board, increase to biweekly.

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 and/or the ISA Technical Board.

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.

1. Surveillance inspections shall consist of visual inspections by an accredited veterinarian, 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 upon request.
2. The accredited site veterinarian will be responsible for personally conducting a minimum of one monthly veterinary surveillance inspection of each respective site for which she/he has fish health responsibility. This should coincide with a scheduled dive to collect mortalities. More frequent surveillance requirements should include veterinary inspections as defined in #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.

C. Sample specifications

Active sites will be sampled as follows:

1. Appropriate numbers of moribund or recently dead fish will be collected per scheduled veterinary surveillance inspection. A maximum of 10 targeted fish per routine monthly site visit should be collected whenever possible or a minimum of 3 within a two month period. 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.
3. Samples will be tested in accordance with diagnostic testing protocols in [Part III D](#) and by USDA APHIS-approved laboratories (See [Part III E](#)).

4. Sample specifications: **Table 1** below lists sample specifications for the ISA assays to be performed.

Table 1: Sample Specifications for ISAV Control Program Approved Assays

TEST	Assay Pool	Tissue	Collecting vessel/ Preservative/media
RT-PCR	Single fish	0.25 cm ³ mid kidney	2 ml tube/RNA Later
IFAT	Single fish	2-3 Mid-kidney impressions	Frosted end slide
Virus Isolation (cell culture)	Up to 5 fish per pool per net-pen	Kidney, spleen, heart	Specimen cup/approved media

D. Diagnostic Tests and Diagnostic Testing Standards

The official diagnostic tests for the ISAV Control Program include the following:

- a) RT-PCR
- b) IFAT
- c) Virus Isolation (cell culture)
- d) Sequence
- e) Gross Pathology
- f) Other approved tests

All official tests for ISAV will be performed by an approved laboratory, except for gross pathology which will be evaluated by the accredited veterinarian or USDA-authorized sampler. The laboratory will use ISAV diagnostic test procedures that have been reviewed and approved by APHIS’ National Veterinary Services Laboratories.

Surveillance of all sites will utilize RT-PCR as the primary screening diagnostic test, and IFAT as a secondary assay. IFAT impression smears will be obtained from all sites and will be acetone-fixed and archived at the receiving laboratory. IFAT slides corresponding to any tissue sample testing positive by RT-PCR will be subsequently processed and evaluated.

Tissue samples for virus isolation should be collected during inspections of a Category 2 or higher status site, or at the discretion of the accredited veterinarian, the Program Veterinarian and/or the ISA

Technical Board. All suspect and infected tissue samples and positive supernatants will be archived (for at least a 1 year) by the receiving laboratory after testing has been completed.

A positive RT-PCR is considered suspect, and requires immediate notification of the ISA Program Veterinarian, Maine DMR the accredited veterinarian and the National Veterinary Services Laboratories (NVSL), the APHIS VS reference laboratory. Tissue samples of initial suspect cases will be sent to NVSL from the originating laboratory within 24 hours of suspect results for official confirmatory testing. RT-PCR assays will be sequenced as part of this testing at NVSL.

Resolution of inconclusive or conflicting diagnostic tests will be determined by the USDA APHIS ISAV Control Program Veterinarian or the District 1 DD or AD (or delegate) following consultation with the applicable accredited veterinarian, the approved laboratory, and NVSL. If a sample or site status remains unresolved after consultation then referral to the ISA Technical Board is required. Issues surrounding conflicting or otherwise questionable results may be resolved through the following (independently or in combination):

1. Additional tests performed on archived samples by the approved laboratory.
2. Additional tests on archived samples performed by the NVSL
3. Collection of additional samples from the site or net-pen and with testing by the approved laboratory and/or NVSL.
4. Increased sampling frequency from the net-pen in question, additional net-pens, or net-pen site.
5. Consultation with the ISA Technical Board.

The Program Veterinarian and District 1 AD (or delegate) shall consider recommendations made by the ISA Technical Board and will make final determination (in conjunction with the Maine DMR) on all ISAV Control Program issues.

E. Biosecurity audits

All marine sites and vessels will be subject to third party biosecurity audits at least once a year, in addition to any internal audits. Records shall be kept of these audits, including recommendations for improvement and any corrective steps taken to address deficiencies.

1. Marine sites that are classified as ISAV Control Program Site Category 2-5 shall be subject to more frequent biosecurity audits.
2. If a processing plant is receiving fish from Category 2-5 sites, they may be subject to periodic biosecurity audits.

F. Reporting

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.

All surveillance reports and actions regarding ISA control measures must reference the site identification code and name. All surveillance documents, laboratory reports, and other documents as required will be forwarded to the Program Veterinarian. A flow chart reflecting the reporting mechanism is provided in [Appendix D](#). Reporting responsibilities and requirements are as follows:

Accredited Veterinarians: Accredited veterinarians shall, in accordance with *Title 9 Code of Federal Regulations Part 161.3*, report to the Program Veterinarian and the Maine DMR any suspected or confirmed cases of ISA within 24 hours of learning of these test results or diagnosis. Accredited veterinarians shall submit all diagnostic samples for ISA to an approved laboratory in accordance with instructions provided by these Standards, the Program Veterinarian, or the District 1 AD (or delegate), and shall follow any and all procedures as instructed by them. Accredited veterinarians shall use either USDA APHIS Specimen Submission Form (VS form 10-4) or other approved form developed by the Program Veterinarian (such as the included [Sample Submission Chain-of-custody](#) form).

Approved Laboratories: The approved laboratory shall report results of surveillance screening tests to the Program Veterinarian, the District 1 AD (and/or delegate), the Maine DMR and the submitting accredited veterinarian within 10 days of sample receipt. In the case of a suspect or positive result, those listed above will be notified within 24 hours of test completion.

The laboratory shall report *all* test results for ISAV sample collections within 24 hours of testing completion to the Program Veterinarian, the District 1 AD (or delegate), the Maine DMR, and to the submitting accredited veterinarian. If viral culture was included in the sampling, the final report of

sample results must be submitted to the same entities as above within 30 days from the date of sample receipt. A flow chart reflecting the reporting mechanism is provided in [Appendix D](#).

Part IV. Standards for Disease Control Actions and Management

A. ISAV Control Program Categories

ISA Program Categories and their descriptions are found in **Table 2** below. The purpose of the classification system is to provide the Program Veterinarian and Technical Board more information for sites so that further evaluation, epidemiological investigation, and testing can be designed, ordered, and initiated as appropriate.

Table 2: ISAV Control Program Categories and Description

Category	Category Description
Category 1 (presumed negative)	HPR-deleted ISAV has not been detected within at least the previous two months in a net-pen or site participating in active ISAV monthly surveillance testing; considered <i>negative</i> for ISA.
Category 2 (suspect)	Any variant of ISAV has been detected in a net-pen or site by at least one diagnostic test in at least one fish; considered <i>suspect</i> and needing further evaluation within 7 days.
Category 3 (infected)	ISAV has been detected in at least two fish from a single net-pen by at least two official diagnostic tests , one of which includes an HPR-deleted sequence result. For subsequent detections in other (previously negative) net-pens at the same site, a net-pen may also be designated Category 3 based on one fish positive by two tests and one fish positive by virus isolation (from a different tissue pool) – both fish must have one HPR-deleted sequence result.
Category 4 (diseased)	As for Category 3 above, plus clinical disease is present (as diagnosed by an accredited veterinarian).
Category 5 (diseased)	As for Category 4 above, plus mortality consistent with clinical ISA is present at the average rate of 0.05% per net-pen population per day over one week.
Category 6	Net-pen or site previously classified as Category 2 through 5 has been fallowed.

Net-pens and sites, owned by producers participating in the ISAV Control Program, will be categorized according to the surveillance test results criteria included in Table 2 above. 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.

Actions to be taken will depend upon site Category and are as follows:

1. **ISA Program Category 1:** net-pens/sites will continue to conduct surveillance at monthly intervals as detailed in [Part III B](#) (Surveillance Requirements).
2. **ISA Program Category 2:** net-pens/sites will be required to undergo follow-up sampling, including individual sample virus isolation, within 7 days of the report of a positive test result in order to ascertain a more precise program category if possible; except in the case of an HPR0 finding. An HPR0 finding will immediately reclassify the net-pen/site as Category 1. Follow-up 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. Based on the particular situation: : 1) the Program Veterinarian will notify the District 1 AD (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 APHIS headquarters staff may occur. If sequencing results in HPR0, Category 2 net-pens/sites will revert to Category 1. In all other cases, Category 2 net-pens/sites will revert to Category 1 after four consecutive negative biweekly veterinary surveillance inspections and/or after consensus by an ISA Technical Board vote in consultation with the Program Veterinarian.
3. **ISA Program Site Category 3, 4 or 5:** When net-pens are declared infected or diseased by the Program Veterinarian, depopulation orders will come from the Maine DMR per their [Chapter 24](#) regulations. A site- specific action plan for the control and management of the disease will be developed by the producer in consultation with the Program Veterinarian and ISA Technical

Board. The Program Veterinarian may request inventory, mortality data, etc. from the affected site.

4. Alternatively or simultaneously, the Maine Commissioner of Marine Resources or a delegate may put any site falling into Category 2 through 5 under restriction or quarantine, if it is felt such action is necessary to prevent the spread of disease or disease agent. For sites under restriction, no additional fish may be transferred to or from the site unless specifically authorized in writing by the District 1 AD (or delegate) and DMR. The site accredited veterinarian will also be informed of any restriction or quarantine measures.
5. The Program Veterinarian 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. Quarantines

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 and materials have been properly cleaned and disinfected. 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 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 and Maine DMR.

C. Indemnity

As stipulated in Title [9 CFR Part 53.10](#), and when funding is available, indemnity payments will be made to producers complying with the Standards, for fish destroyed due to ISA. These may include ISAV-infected as well as ISAV-exposed fish. The District 1 AD (or delegate) or Program Veterinarian will make final determination as to the Category of the site and approval of indemnity payments to producers. A schedule of payments will be coordinated by the Program Veterinarian or the District 1 AD (or delegate).

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.

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. Marketable fish will be paid also based on a per-fish basis using the established indemnity scale with the USDA paying any difference between individual fish salvage proceeds and the established 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 F](#) and [Appendix A](#) of these Standards.

Indemnity payments are based on the availability of funds approved for that purpose.

D. Depopulation

All depopulation orders will arise from the regulatory authority of the Commissioner of the Maine DMR. Depopulation may be ordered based on net-pen or site Program Category or as a result of consultation with the Program Veterinarian or District 1 AD (or delegate), and/or recommendations from the ISA Technical Board. Additional discussions may take place among those parties in case of disagreements, or if clarification is desired.

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 [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 District 1 AD will oversee the depopulation procedures for as many units as may be practical.

E. Permitting

All fish, nets, equipment, and other fomites capable of transmitting ISAV must be permitted for removal from all [Category 2](#) and higher sites. Permitting will be coordinated between USDA APHIS VS and the Maine DMR to allow movement of fish to fish processing plants, fish composting facilities, rendering, or landfill sites.

F. 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 should 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.

G. 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 pathogenic ISAV, fallowing time period begins only upon completion of all cleaning and disinfection procedures of net-pens, 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 to any Category 1 – 5 site	1
Travel from Category 2 site to any other Category 2 – 5 site	1
Travel from Category 3 – 5 site to any Category 1 or 2 site	2 or 3*
Travel from Category 3 - 5 site to any other Category 3 through 5 site	1
Travel from a wharf servicing of vessels coming from Category 2-5 to Category 1	2
Travel from Category 3-5 fish health zone to any Category 1 or 2 fish health zone	2 or 3*

* As determined by DMR and ISA Program Veterinarian

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.

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 should 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 should 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 bio-fouling.
 - 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, 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.
- Mortalities should be removed from the site after each dive, or as soon as possible.
- 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.
- 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.

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.
- 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.
- 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.
- Empty mort containers as soon as possible, preferably after each mort dive.
- 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. Refer to DMR [Chapter 24](#) regulations.
- Eggs and juvenile stages at fresh water facilities must never 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: Guidance for laboratories and VS personnel in the event infectious salmon anemia virus (ISAV) is detected at a Maine Atlantic salmon production facility

1. Within 24 hours of any ISAV detection:
 - a. The diagnostic laboratory will notify the sample submitter, facility accredited veterinarian (if different from submitter), and the APHIS ISAV Program Veterinarian. This should occur before any additional testing is initiated at the receiving diagnostic laboratory.
 - i. The ISAV Program Veterinarian will notify the VS District 1 AD and Fish Biologist of the detection.
 - ii. The District 1 AD will notify the VS District 1 DD and the APHIS AQ Program Leader.
 - In all cases, confirmation of receipt of notification is requested or to provide contact for alternate representative.
 - iii. The ISAV Program Veterinarian or fish biologist will notify Maine DMR contact. The State may enact any ‘hold’ order they deem necessary pending further information.
 - b. The diagnostic laboratory will notify the NVSL of the positive detection and ship appropriate samples for confirmation. This should occur before any additional testing is initiated at the diagnostic lab.

In general:

- i. **Priority 2** status will be assigned for cases of PCR + findings in healthy animals [no mortality or morbidity associated with case] found on routine surveillance samples
 - ii. **Priority 1** status will be assigned:
 - For cases of PCR + findings in animals showing clinical signs consistent with infection with ISAV and/or high mortality
 - For samples from animals showing clinical signs consistent with infection with ISAV and/or high mortality, sent directly from the field and not previously tested
 - When ISAV HPR-deleted is suspected in in apparently healthy animals [no mortality or morbidity associated with case]
2. The ISAV detection information will be entered into the National Aquatic Animal Instance of SCS (Surveillance Collaboration Services) database and EMRS (Emergency Management Response System), along with any relevant attachments.
 - a. In EMRS, an FAD investigation will be initiated and an FAD # assigned
 3. If the initial detection of ISAV is not confirmed at the NVSL, meaning the NVSL reports negative findings for ISAV in the submitted sample(s), the EMRS and SCS databases will be updated and the EMRS investigation will be closed out (new information may cause the

case to be re-opened).

4. If ISAV HPR0 is confirmed at the NVSL:
 - a. All those mentioned above will be immediately notified of confirmation.
 - b. The Aquaculture Program Leader, or a representative, will notify the VS international health standards services group for discussion and a decision will be made on reporting to the OIE.
 - i. Generally HPR0 detections are included in the APHIS OIE 6 month report. This report is simply that it occurred during the 6 month reporting time frame.
 - c. The ISA Technical Board will be notified by the ISAV Program Veterinarian or Fish Biologist. The FAD investigation will be updated and closed out in EMRS.

 5. If ISAV HPR-deleted is confirmed at the NVSL, in even a single fish,
 - a. All those mentioned above will be immediately notified of confirmation
 - b. The Aquaculture Program Leader, or a representative, will notify the VS international health standards services group for discussion and a decision will be made on reporting to the OIE.
 - i. The Maine DMR will notify other State agencies they deem applicable. The State may enact any ‘hold’ order they deem necessary pending further information.
 - c. Follow-up samples will be collected by the facility Accredited Veterinarian or other appropriate designee and duplicate samples sent to both the NVSL and diagnostic laboratory.
 - i. For marine farms, within *7 days* of the confirmation notification (as per the Program Standards)
 - ii. For freshwater facilities, within *3 days* of the confirmation notification
 - d. The ISA Technical Board will convene to discuss next steps. For marine farms, the Program Standard protocols will be followed. For freshwater facilities, similar protocols may be discussed and decided upon.

 6. The incident will be updated in EMRS as an investigation (non-confirmed HPR-deleted) or FAD (confirmed HPR-deleted). It will be closed out once the incident has been resolved.
-

APPENDIX C: ISAV Control Program Contacts

USDA APHIS VS SPRS:

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[Navigation Notes](#)

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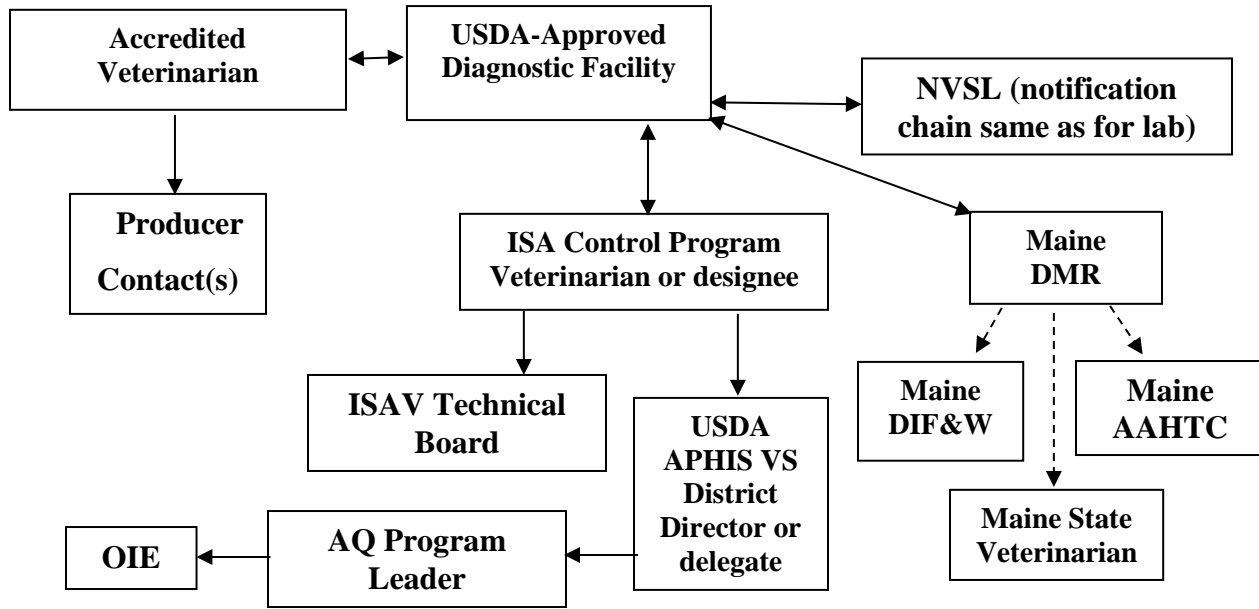
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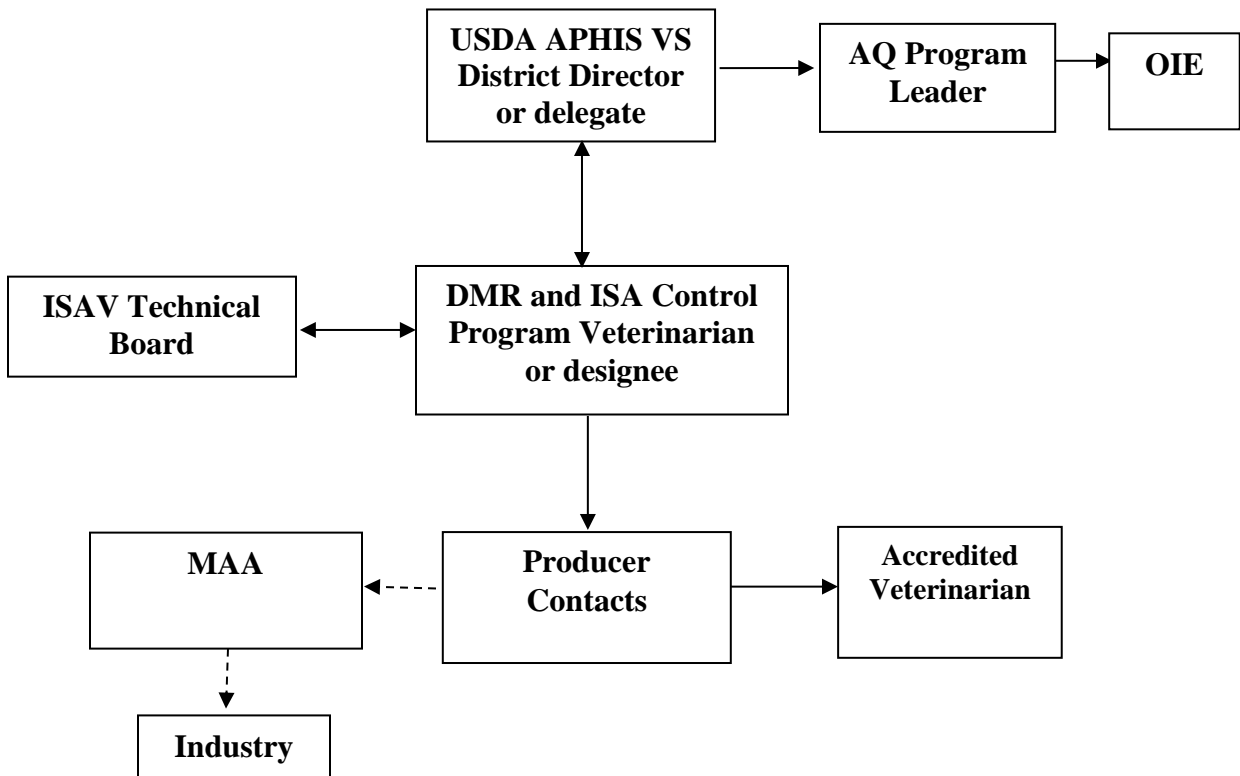
APPENDIX D: USDA APHIS ISAV Control Program Flow Charts

Non-negative Notification Flow Chart



----> In the case of confirmed infection notify these entities

Action Notification Reporting Flow Chart



[Navigation Notes](#)

APPENDIX E: 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

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