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# Infectious Salmon Anemia Program Standards

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**USDA APHIS Veterinary Services**

**Maine Department of Marine Resources**

**Maine Aquaculture Association**

Version January 2010

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The **United States Department of Agriculture, (USDA), Animal and Plant Health Inspection Services, (APHIS), Veterinary Services, (VS) *Infectious Salmon Anemia Program Standards, (ISA)***, hereafter referred to as the “*Standards*”, establishes recommended procedures for the prevention and containment of Infectious Salmon Anemia (ISA) from farm raised Atlantic salmon. These standards provide industry, accredited veterinarians, approved laboratory personnel and regulators a template to follow to meet the goals of this Federal program. Indemnity payments, an integral part of this program, may be made to producers at a set schedule provided established procedures and standards described herein are followed, and provided that adequate funding is made available. It is further understood that these standards must be open to amendment as science and circumstances evolve. 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 on ISA. This current version of the Standards resulted from recommendations put forward by the ISA Technical Board through the end of 2009.

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## Introduction

The agent responsible for ISA is a single stranded, enveloped RNA virus composed of 8 genomic segments. It is related to the influenza viruses but belongs to a distinct genus, *Isavirus* (ICTVdB Index of Viruses), within the family Orthomyxoviridae. Viral particles are spherical with a diameter ranging from 45 to 140 nm (Dannevig 1995). Intracytoplasmic inclusions of 2.5 µm have been observed in infected endothelial cells. The presence of a hemagglutinin capable of binding erythrocytes of Atlantic salmon, rainbow trout, cod, and carp is believed to facilitate binding and infection of host cells (Falk et al. 1997). The virus attacks endothelial cells, producing hemorrhage in affected fish (Nylund et al. 1995). Variable gross lesions may include ascites, petechiae along the GI tract, in mesenteric fat, on the swim bladder and kidney capsule, as well as congestion and necrosis in the liver, spleen and kidneys. As its name implies, ISA causes anemia. Hematocrits in fish showing clinical disease may range from near normal to as low as 2 or 3%. Outwardly, affected fish may also show a variety of associated signs including pale gills, exophthalmia or bulging eyes (with or without hemorrhage), lethargy, and darkened skin surfaces (Evensen et al. 1991).

Distribution of the ISA virus (ISAV) is widespread. The virus has affected fish in Norway, Scotland, the Faroe Islands, New Brunswick, Canada, the Cobscook Bay area of Maine, and Chile. Byrne et al. (1998) describes a hemorrhagic kidney syndrome in Atlantic salmon from the east coast of Canada which appears in retrospect to be the first account of ISA in North America.

ISAV may have evolved from among wild populations in North America as is believed to be the case in European populations (Plarre et al. 2005). Sequence comparisons of ISAV isolates have identified two general subtypes, one termed North American and the other European (Kibenge et al. 2001; Krossøy et al. 2001); molecular studies on isolates from Norway, Scotland, and Canada, appear to indicate that these two subtypes diverged more than 100 years ago (Krossøy et al. 2001). Genotype variation within the subtypes is evident, and apparent pathogenicity varies from non-virulent to virulent (Ritchie et al. 2001; Mjaaland et al. 2005; Kibenge et al. 2006). In Maine, the first genotype of ISAV that was isolated was determined to be identical to the pathogenic New Brunswick genotype of virus described by Mullins et al.

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(1998). In subsequent years, additional pathogenic genotypes were detected at sites in both Maine and New Brunswick (Micro Technologies Inc. & New Brunswick Department of Agriculture & Aquaculture, pers. comm.). A second genotype of ISAV (i.e. HPR0 genotype) that does not appear to cause pathology to date, clinical disease or increased mortality has been repeatedly detected by RT-PCR since 2003 at many sites in Maine and New Brunswick (Micro Technologies Inc., pers. comm.; Cook-Versloot et al. 2004). Since the apparently non-pathogenic genotype is not currently detectable by IFAT, has not been successfully cultured to date and currently does not produce pathology or mortality, its detection solely by RT-PCR, with confirmation by genomic sequencing, does not automatically trigger disease control action parameters. Nevertheless, the increased complexity of multiple genotypes, differing impacts and regulatory responses have mandated the addition of regular sequencing protocols to the diagnostic process. As new discoveries regarding different genotypes and their pathogenicity are made, so will our view of which genotypes are pathogenic and which are apparently non-pathogenic.

The ISA virus is transmitted mainly through viral particles shed in fish mucus, feces or tissue wastes, or through direct contact with other infected fish (Rolland & Nylund 1998). Epidemiological investigations indicate that the virus is also spread through untreated waste products and water coming from harvest operations, natural circulation of water between infected and uninfected sites, fish processing plants, and shared equipment and gear that has not been properly disinfected at marine sites (Vagsholm et al. 1994, Jarp & Karlsen 1997, Jarp 1999, McClure et al. 2005, Hammell et al. 2005, Gustafson et al. 2005, Ellis et al. 2006). While there is no validated evidence to indicate that ISA virus is vertically transmitted (i.e. from parent to offspring; Melville & Griffiths 1999), an investigation of ISA in first-feed Atlantic salmon fry raises the possibility of infection at very early life stages (Nylund et al. 1999). ISA can also be transmitted indirectly. Sea lice (*Lepeophtheirus salmonis* and *Caligus* sp.) are potential pathogen vectors and may increase disease susceptibility through added stress on fish, which may result in substantial infection and mortality (Rolland & Nylund 1998).

Significant morbidity and mortality due to ISAV are specific to Atlantic salmon held in marine net pens, although experimentally the virus can infect and replicate in a variety of species, including herring (Nylund et al. 2002), rainbow trout (Nylund et al. 1997), and brown trout

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(Nylund et al. 1995),. While the segment of the ISA viral genome corresponding to a primer used in RT-PCR assays has been detected in wild Atlantic salmon, neither the presence of replicating virus nor mortality have been documented in wild free-ranging Atlantic salmon. (G. Olivier, DFO Moncton NB, Canada; P. Barbash, USFWS, Lamar Fish Health Center-- pers. comm.). A variety of marine species may act as ISA virus carriers or reservoirs capable of infecting cultured fish. Although no disease transmission has been documented, experimental laboratory challenges have demonstrated the potential of viral infection from brown trout (*Salmo trutta* L.) to Atlantic salmon through cohabitation (Nylund & Jakobsen 1995; Rolland & Nylund 1998).

ISA virus does not infect humans or other mammals, since the virus is inactivated at temperatures below the typical mammalian internal temperatures of 37°C- 40°C (Falk et al. 1997). The virus is also inactivated by external heat, extreme pH changes and disinfectants containing chlorine or iodine compounds (Torgersen 1997; Torgersen 1998; Smail et al. 2004; NB-DAFA/Washburn & Gillis report 1998).

Experience with the disease at marine sites in Europe and North America indicates that, if uncontrolled, the virus spreads variably from site to site. Anecdotal observations indicate that onset of disease may be extended by several months in marine netpens, depending on the length of time the fish have been in saltwater, water temperature, vaccination status, sea lice numbers, nutrition, site management procedures, stress placed on the fish by environmental or management conditions, and the status of the fishes' immune system. Cumulative mortality due to the disease varies greatly from a very low percentage (near zero) to more than 50% of the fish population. Strategic management practices, including aggressive depopulation of affected cages, supported and facilitated by an effective indemnification program, have reduced actual and potential losses due to ISA (Sandi McGeachy/DAFA, Nell Halse/NBSGA; pers. comm.). Ongoing efforts regarding ISA management at US and New Brunswick farms associate swift detection/removal of infected cages with a consequent reduction in disease spread at an affected site (Gustafson et al. 2006), compared to sites where other (or slower) management approaches are taken (McClure et al. 2004 ). In the United States, clinical disease from ISA has been confined to Cobscook and Passamaquoddy Bays, the latter being an international body of water shared by Maine and New Brunswick, Canada. It has also been

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documented off nearby Campobello Island (New Brunswick) sites as well as in sites located along the International Passage abutting Deer Island in New Brunswick.

## **Major ISA Program Components**

The guiding principles for ISA management, as for most infectious diseases, include aggressive surveillance, risk identification, reduction and avoidance, strict biosecurity, selective depopulation, and site fallowing. 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 appears to be an achievable goal. Information from Norway, Scotland, and North America has shown that the prevalence and spread of disease among cages and sites, and the number of fish lost to disease, can be variably controlled through the development and deployment of effective management techniques. These include strict biosecurity and disinfection practices, selective age-grouping by location, the use of site-specific personnel and equipment, and ongoing surveillance programs coupled with aggressive depopulation of affected fish cages. Fish health regulatory agencies in other countries, and many companies through their Best Management Practices, have developed similar approaches to disease management for ISA.

The farmed salmon industry in Maine and maritime Canada uses highly qualified personnel experienced in all aspects of fish culture, husbandry, and health management on both sides of the international border. Most industry members have established in-house procedures for increased disease surveillance and a working relationship with aquaculture veterinarians and diagnostic laboratories to provide further technical expertise. The industry complies with mandated regulatory fish health inspections to meet import and export regulatory requirements for fish. Current requirements in Maine include health inspections for smolts before transfer from freshwater hatcheries to marine sites and lethal testing of broodstock at spawning. Additional elective diagnostic sampling is also conducted to meet individual company management protocols and production requirements.



The industry has worked with academia and regulatory agencies to establish biosecurity protocols for aquaculture operations. Exposure to ISA risks has been assessed through a series of biosecurity audits conducted over the last 8 years by both independent auditors as well as through internal company audits. Audits were initiated in 2001 to assess biosecurity and the risk of disease transmission through shared equipment such as boats traveling between sites in Canada and the United States or among United States' sites. Fish Health Zones were previously established in Maine Department of Marine Resources (DMR) regulation. With the detection of ISA virus and disease in Maine an ISA Increased Risk Zone designation was established in February 2001 by DMR for Cobscook and Passamaquoddy Bays. In the event that additional areas in Maine experience clinical infection by a pathogenic genotype of ISAV, and/or develop disease, the Increased Risk Zones will be modified accordingly. To insure that objectives for ISA control are consistent across the industry, DMR established regulations to define the ISA clinically diseased areas, require mandatory ISA virus surveillance, and restrict finfish aquaculture vessel and equipment movement. In addition, these ISA Standards were developed to specify the testing procedures to confirm ISA virus, stipulate chain of custody requirements, standardize sampling requirement protocols, identify responsible inspectors, and stipulate consequent action upon detection of ISA virus. The agency has incorporated these emergency measures into the DMR [Chapter 24](#) Regulations (see section 24.21).

In January of 2002, the Maine farmed salmon industry developed a Bay Management Program encompassing single year-class stocking, site allocation, and strategies for the transfer of fertilized eggs (and subsequent fingerlings) and smolts. In view of the uncertainty surrounding potential vertical transmission of ISA virus, the aquaculture industry has voluntarily adopted precautionary measures that apply to many areas of fish husbandry to limit this risk, including broodstock screening for ISA virus and thorough egg disinfection protocols at fertilization and water hardening. Due to the shared water resources, as well as shared personnel and equipment by companies raising fish in both New Brunswick and Maine, coordination of ISA management programs between New Brunswick, Canada, and Maine is considered essential and is an ongoing focus of attention by the regulatory authorities of both countries.

In summary, this federally sponsored ISA program consists of seven components, which include surveillance, testing, disease reporting, disease control and biosecurity, quarantine,

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depopulation, and indemnity (subject to availability of funding). Surveillance ensures that resources and producers' attention will be directed at routine and regularly scheduled inspections and health assessments of fish so that ISAV infection will be quickly diagnosed when present. Testing with the best and most scientifically sound assays at an USDA 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, also elements required for this program, will also mitigate pathogen and disease spread. Prompt depopulation of cages of infected fish eliminates their continuing transmission risk. Finally, indemnity, when available, provides financial relief to producers while encouraging prompt reporting and compliance with all of the ISA Program Standards.

## Part I. Definitions

**Accredited veterinarian:** a veterinarian holding a current state veterinary license who has also fulfilled the accreditation requirements of United States Department of Agriculture Animal and Plant Health Inspection Service (USDA, APHIS).

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

**Active site:** a finfish lease site as designated by the Maine DMR, and at which fish are present.

**Approved fish processing plant:** a fish processing plant that is approved by the USDA APHIS ISA Program Veterinarian, undergoes audits for biosecurity and treats waste-water and blood water prior to discharge into surrounding waters to sufficiently inactivate any ISA virus that may be present.

**Approved laboratory:** a laboratory that is approved, by USDA APHIS, to conduct official diagnostic tests for ISA virus (Reference VS memorandum 567.2).

**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 in hatcheries or other freshwater facilities, or in marine net-pens.

**AVIC:** the USDA APHIS, Veterinary Services, Area Veterinarian in Charge responsible for the administration and management of federal animal disease programs in states.

**Biosecurity:** procedures designed to eliminate or lessen physical or economic losses involving farmed stocks. Risks of acquiring or transmitting pathogens are assessed and factored into a comprehensive program involving aspects of site design, stock selection, and husbandry

practices, therapeutants, veterinary supervision, and many other management variables (See [Appendix A<sup>2</sup>](#)).

**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](#)).

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

**Broodstock:** reproductively mature fish that have been selected or used as a part of a defined breeding program and designated as broodstock no earlier than month 28 post-introduction into sea water. 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. .

**Carryover:** fish from a previous year class held on a site after the subsequent year class has been placed on that site.

**Cell culture:** *See Virology.*

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

**CPE:** cytopathic effect, or the observable disruption of a cell culture monolayer by a virus. Many viruses have specific types of CPE, which may be confirmed through RT-PCR, using pathogen-specific primer sequences.

**Depopulation:** removal of all fish of a defined fish population within a cage, lease site or other venue with the intent of eliminating a disease outbreak.

**Dipnet:** apparatus for removing moribund or dead fish from the surface water of cages.

**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.

**Diseases of Regulatory Concern:** infectious diseases that have 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:** Maine Department of Marine Resources.

**Fallow:** complete depopulation of all ISAV-susceptible cultured aquatic organism populations within a marine site or defined management zone. Fallowing is coupled with cleaning and disinfection protocol of the depopulated area. The levels of cleaning and disinfection are established in [Part III: Disease Control Actions and Management](#).

**Fallowing time:** the period of time following the complete emptying, cleaning and disinfection of an active cage or site, and prior to restocking. The start, end and duration of the fallow will be determined by the ISA Program Veterinarian. See also [Part III: Disease Control Actions and Management](#).

**Fish Health Zones:** defined marine geographic areas as designated in Maine DMR [Chapter 24](#) regulations (see section 24.01).

**Genotype:** A group of evolutionarily related viruses possessing a defined degree of nucleotide sequence relatedness (The defined degree of relatedness will vary by virus and gene or region of genome being compared).

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**Genotyping:** Determination of the genetic similarity of a particular ISAV isolate to described ISAV isolate groupings that are based on variations in genomic sequences targeted, such as partial sequences from the segment 6 HPR region or segment 8.

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

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

**Horizontal transmission:** infection spread from one fish to another through means including close physical proximity that facilitates contact with infected tissues or products, contaminated equipment, sea lice, untreated processing plant effluent, infected wild fish and other mechanisms.

**Incubation:** period of time between infection with ISAV and the appearance of clinical signs; generally thought to be around 3 weeks but which may be variable depending on other factors.

**Indemnity:** compensation paid to farmers in exchange for depopulation of a fish population.

**IFAT:** indirect fluorescent antibody test; an assay that makes use of 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. Detection of the pathogen is based on evaluation of the resulting fluorescent patterns.

**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.

**Industry:** All entities involved in the production of marketable farm-raised Atlantic salmon, including freshwater hatchery owners or lessors, marine lease-site holders,, and processors operating in Maine.

**Inspection:** an on-site visit and/or a statistically-based sampling of fish on the facility and the resulting laboratory tests and inspection reports conducted by an inspector in accordance with the testing requirements and procedures set forth in this plan.

**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.

**Introduce:** to import, or to land on, bring into or deposit in any place subject to the jurisdiction of the State of Maine from any restricted areas within the State of Maine.

**ISA:** infectious salmon anemia; the clinical disease resulting on a variable basis from infection by a pathogenic genotype of ISA virus. Signs include hemorrhage and anemia, which may result in very pale gills or reduced hematocrit. Other external signs may include exophthalmia, lethargy, epithelial darkening, petechiae (pinpoint hemorrhages) on the skin, or nothing at all. Sero-sanguinous fluid accumulations (i.e. ascites) are often observed in peritoneal and pericardial cavities. Internally, surfaces of kidneys, livers, intestines, or swim bladders may show signs of petechiae or hemorrhage. Mortality due to ISA varies.

**ISAV:** Infectious salmon anemia virus; an infectious pathogen with numerous genotypes, some of which are capable of infecting several species of finfish. Morbidity and mortality have only been documented in *Salmo salar*.

**ISA Program Site Categories:** staged categories of ISA status at marine pen sites following a review of official diagnostic tests by the ISA Program Veterinarian in conjunction with input from the ISA Technical Board if necessary. The ISA Program Site Categories are defined in [Table 2](#).

**ISA Program Veterinarian:** USDA APHIS VS veterinarian assigned to manage the ISA Program for USDA APHIS VS in Maine, and who reports to the Area Veterinarian in Charge for Maine.

**ISA Risk Zone:** a Fish Health Zone or subsection classified with respect to ISA risk, based upon epidemiological data and analysis.

**ISA Technical Board:** a group of five people consisting of the USDA APHIS ISA Program Veterinarian, one Maine DMR representative and 2 industry representatives, plus a nominal chairperson selected by the committee representatives. The duties of the board are to make recommendations to the ISA Program Veterinarian, the USDA APHIS Area Veterinarian in Charge (AVIC) and/or Maine DMR Commissioner after being called together to consider positive lab results, epidemiological data, audit reports or other information regarding reported disease risks or conditions requiring action under the terms of this program. A quorum will be all four voting representatives. The chairperson only votes in the case of a tie and acts as a facilitator.

**Negative site:** an active site testing negative for ISAV while involved with active participation in an official surveillance program, including inspection by a veterinarian (See ISA Case Definition [Category 1](#)).

**Netpens:** also called “cages”; plastic or steel structures of differing sizes and shapes designed to contain variable numbers of fish.

**Non-pathogenic:** not capable of inducing disease (i.e. no clinical signs/gross pathology observed and no increased mortality in a population).

**Official Diagnostic Test:** procedures for the detection of ISAV performed by a USDA APHIS VS approved laboratory: including 1) Indirect Fluorescent Antibody Test (IFAT), 2) Gross pathology, 3) Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and 4) Virus isolation.

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**Owner:** any person, partnership, company or corporation with a proprietary interest in the fish culture facility being inspected.

**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:** capable of causing disease (i.e. clinical signs/gross pathology observed and increased mortality in a population).

**Permit:** a USDA APHIS Veterinary Services document entitled Permit for Movement of Restricted Animals (VS form 1-27) which allows movement of animals from ISA infected or suspect premises to insure biosecurity.

**Processing plant:** any facility where Atlantic salmon (including products such as fillets used for value-added purposes) are taken for processing or rendering into a marketable product.

**Production fish:** fish destined for market or harvest.

**Qualified source/hatchery:** an established source/hatchery that has had 3 consecutive annual inspections in which pathogens as described in Maine State Regulations [Chapter 24.21](#) (subsection D) have not been detected; or a new hatchery that has had 3 successive negative annual inspections over a continuous 2 year period.

**Quarantine:** no movement of live fish to or from the site except for processing or slaughter as approved and permitted by USDA or the Maine DMR. In addition 1) no visitors to site except necessary fish health personnel; 2) institution of a biosecurity program approved by the Commissioner of Maine DMR; and 3) disposition of deceased and quarantined fish in a manner approved by the DMR Commissioner.

**Restriction:** no movement of live fish off or to the site and institution of a biosecurity program approved by the Commissioner of Maine DMR.

**RT-PCR:** reverse-transcriptase polymerase chain reaction, a process where RNA or DNA is multiplied in a cyclic fashion using a special, sequence-specific primer that binds to the genome of a particular organism if it is present in even extremely small numbers. The resulting amplification of the RNA or DNA of that organism is made visible by means of photographing a stained gel in which the amplified genomic fragments have been separated based on their size.

**Sampler:** a person designated by an accredited site veterinarian for the collection of surveillance and diagnostic samples at the direction of the veterinarian, and using an established chain-of-custody document for the respective sample. Samplers will be trained by an accredited veterinarian and approved by the USDA APHIS ISA Program Veterinarian. Identification of the sampler must be referenced in all documents related to surveillance activities at a site. The sampler's role is limited to completing the complement of required surveillance submissions and/or processing samples for submission.

**Sea lice:** copepod arthropods belonging to either the *Lepeophtheirus* or *Caligus* genera. Both species infest Atlantic salmon and live in the mucus layer, where they attach and suck blood or cause sores. Studies indicate that *Lepeophtheirus* may be a vector capable of transmitting ISAV to Atlantic salmon (Nylund et al. 1993; Nylund et al. 1994). Both species can cause stress on fish, which adversely affects the immune response.

**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:** a marine site containing only one year-class of salmon at a time. That year-class of fish is entirely removed and fallow implemented prior to introducing salmon of another year class to that site.

**Site:** (or ‘marine site’ or ‘finfish leased site’) a specific leased area in saltwater where fish are raised. Sites in Maine waters must be identified with the specific site identification code issued by the Maine DMR.

**Site identification number:** a finfish aquaculture lease site identification number 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 site.

**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 a pathogen or disease through periodic sampling and testing fish within cages or sites. Programs are generally formulated to establish the presence or absence of a particular pathogen or disease using a statistically defined sampling plan. The sampling plan allows an estimate of prevalence or the extent of either infection or disease in a population within a specified level of probability.

**Transfer permit:** a permit issued by the Commissioner of Marine Resources (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:** infection passing from one generation to another. True vertical infection involves a transmissible virus within the egg or sperm that cannot be eliminated by external disinfection protocols.

**Virology:** (aka ‘cell culture’ or ‘virus isolation’) growth of a virus on a particular cell line that is susceptible to being infected by that virus. In theory, only one virion, or virus particle, is needed for infection, growth, and replication.

**Year class:** grouping of a population of fish of the same age/hatch year and/or grouping by year of saltwater transfer.

## **Part II. Disease Surveillance and Investigation**

### **A. Basic requirements**

An ongoing disease surveillance program to facilitate early detection of both pathogen and disease is essential for effective prevention, management, and control of a wide variety of aquatic diseases including ISA. The following basic requirements for the ISA Program are necessary and mandatory for industry for effective ISA control in Maine:

- a. Each industry member or company with Atlantic salmon grow-out sites in Maine will establish and maintain a veterinary-client-patient relationship with a licensed and APHIS-accredited (hereafter ‘accredited’) veterinarian. The accredited veterinarian will have responsibility for implementing all provisions of the ISA Program Standards at participating sites under his/her supervision.
- b. Accredited veterinarians will conduct surveillance on behalf of their industry clients/leaseholders, and adhere to testing and reporting procedures for ISAV/ISA as described in these Standards.
- c. Individual salmon-producing companies 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](#): Fish Health and Biosecurity Guidelines). In addition to the inputs of these personnel, biosecurity protocols will be periodically reviewed by the USDA APHIS ISA Program Veterinarian.
- d. Each company shall develop a site-specific (and year-class specific, if applicable) ISA action plan for the prevention, control and management of ISA. Involvement of the company or service veterinarian and fish site health manager is essential. Site-specific ISA action plans will be reviewed as necessary by the USDA APHIS ISA Program Veterinarian.

- e. Industry participation in the Integrated Pest Management Program for the Control of Sea Lice on Salmonids in Maine is mandatory (see [Appendix C](#): Integrated Pest Management) as studies have indicated that *Lepeophtheirus* sea lice may be a vector capable of transmitting ISAV to Atlantic salmon (Nylund et al. 1993; Nylund et al. 1994).
- f. Industry will submit to the USDA APHIS ISA Program Veterinarian fish inventory information for every leased finfish site with site and cage identifiers. Fish inventory information must include the numbers, age, hatchery of origin, date of saltwater transfer, vaccination status, and previous therapeutant history for all fish in the unit. This will provide APHIS VS with data necessary to establish disease control actions, complete epidemiological assessments, and increase the ability to effectively monitor fish populations.
- g. Industry will maintain and make available to USDA APHIS VS mortality data for each site and pen in production. This can be accomplished utilizing existing industry records systems and log sheets.
- h. Sites will cooperate and assist USDA APHIS VS with the completion of biosecurity audits at marine sites, processing plants, and vessels involved in salmonid aquaculture (See [Part II F](#)).
- i. Documentation required in items c, d, e, and f, above must be submitted to the ISA Program Veterinarian.

## **B. Surveillance Requirements**

Participation in the ISAV surveillance protocol as detailed in this section is mandatory for all active salmonid lease sites to become and remain eligible for indemnification under the ISA program in the event that funds become available. Minimum surveillance activities will involve onsite inspection by an accredited veterinarian or technician under his/her

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supervision, as well as collection of appropriate samples for ISAV assays. In addition, USDA APHIS personnel under the direction of the ISA Program Veterinarian may collect samples as needed as part of this surveillance. The schedule of veterinary surveillance inspections depends upon each site's specified ISA Program Site Category (See Part III. A. [Table 2](#) for Category Definitions). This schedule will be as follows:

**Monthly:** Sites meeting the criteria for ISA Program Site Category 1 in either the low or high risk zone.

**Biweekly:** Sites meeting the criteria for ISA Program Site Category 2 as well as all other marine sites within the same bay which are considered potentially exposed. Also, if a pathogenic detection of ISAV has been made in the lower end of New Brunswick's ABMA1 (around Deer Island or Campobello Island), all Maine sites in Cobscook & Passamaquoddy Bays will increase sampling to biweekly.

**Weekly:** Sites meeting the criteria for ISA Program Site Categories 3 thru 5.

**As Needed:** The inspection schedule will be increased to resolve issues of ISA site category. Sampling frequency may also be adjusted as needed at the discretion of the ISA Program Veterinarian and/or the ISA Technical Board.

**Note:** New sites, ISA Program Site Category 6 sites, or inactive sites returning to active sites must initiate surveillance activities within 30 days of introduction or transfer of salmonids to the site.

1. Veterinary surveillance inspections shall consist of visual inspections of all cages 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 ISA Program Veterinarian.
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. Fish samples for ISA Program testing as described in [Part II D](#) may be collected by the accredited veterinarian or a USDA APHIS VS authorized sampler at the direction of the site

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veterinarian. More frequent surveillance requirements as outlined above or at the recommendation of the ISA technical board or ISA Program Veterinarian should include veterinary surveillance inspections as defined by the ISA program standard definitions.

3. USDA APHIS VS-authorized personnel may also conduct sampling as necessary to comply with these program Standards. This sampling option will be coordinated by the ISA Program Veterinarian after consultation with the producer and their 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**

Sampling and diagnostic evaluation of fish tissues will be accomplished in conjunction with veterinary surveillance inspections conducted as above. In cooperation with producers' schedules, divers, and timeliness for sample submission, sites will be sampled as follows:

1. Appropriate numbers of moribund or recently dead fish will be collected per scheduled veterinary surveillance inspection. A minimum of 10 targeted fish per site should be collected whenever possible. Moribund may be interpreted to include slow-swimming, 'pinhead' (stunted or dwarf fish), otherwise deformed, dark or exophthalmic fish.
2. Samples are to be collected by an accredited veterinarian or technician under his/her supervision, state inspector, federal inspector, or laboratory contract personnel as appropriate. Chain-of-custody documentation on fish samples submitted for ISA Program testing under the surveillance program must be submitted to the USDA APHIS ISA Program Veterinarian.
3. Samples will be tested in accordance with diagnostic testing protocols in [Part II D](#) and by USDA APHIS-approved laboratories (See [Part II E](#)).



4. Sample specifications: **Table 1** below lists sample specifications for the ISA assays to be performed: the number of fish per pool, the required tissues, and the collection technique used for each test.

**Table 1: Sample Specifications for ISA Program Approved Assays: Fish Pool, Tissues and Collection Vessel**

TEST	Fish Pool	Tissue	Collecting vessel/ Preservative/media
RT-PCR	Single fish	0.25 cm <sup>3</sup> mid kidney	2 ml tube/RNA Later
IFAT	Single fish	2-3 Mid-kidney impressions	Frosted end slide
Virology	Single fish/cage (up to 5 fish per pool)	Kidney, spleen, heart	Specimen cup/ PBS

## D. Diagnostic Tests and Diagnostic Testing Standards

To insure that surveillance and diagnostic procedures are standardized throughout the industry the following diagnostic tests will be considered official. The official diagnostic tests for the ISA Program include the following tests:

- a) IFAT
- b) Gross pathology
- c) RT-PCR
- d) Virus Isolation

All official diagnostic tests will be performed by a USDA APHIS-approved laboratory. ISA diagnostic test procedures will be performed according to the protocols established and filed with USDA APHIS VS.

Surveillance will utilize RT-PCR as the primary screening diagnostic test. IFAT impression smears will be acetone-fixed and archived. IFAT slides corresponding to any tissue sample testing positive by RT-PCR will be subsequently tested. Tissue samples for virus isolation should be collected during inspections of a Category 2 or higher numbered site at the discretion

of the accredited veterinarian, the Program Veterinarian and/or the ISA Technical Board. All tissue samples and positive supernatants will be archived (for at least a year) after laboratory testing has been completed. At least the initial positive RT-PCR from each cage will be genetically sequenced. Additional or subsequent diagnostic tests on archived samples will be determined following consultation among the USDA APHIS ISA Program Veterinarian, the approved laboratory, NVSL, and the site accredited veterinarian.

On a first-time basis, samples testing positive for ISAV from previously negative sites (i.e. ISA Program [Category 1](#) sites) by any USDA APHIS-approved laboratory other than the National Veterinary Services Laboratories (NVSL) in Ames, Iowa will be sent to NVSL for confirmatory testing. Archived original tissues should be submitted to NVSL for this confirmation.

Resolution of inconclusive or conflicting diagnostic tests will be determined by the USDA APHIS ISA Program Veterinarian following consultation with the site accredited veterinarian, the approved laboratories, and NVSL. If a sample or site status remains unresolved after consultation with these bodies then referral to the ISA Technical Board is required. Issues surrounding conflicting or otherwise questionable results may be resolved through the following (independently or through combinations):

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

The ISA Program Veterinarian shall consider recommendations made by the ISA Technical Board and make final determination on all ISA Program issues.

## E. Approved laboratories

All ISA program tests must be conducted at USDA APHIS-approved laboratories. Approved laboratories must report all positive test results within 24 hours of completion to the ISA Program Veterinarian, Maine DMR and the accredited veterinarian. All reports must reference all information required in [Part II G](#) (Reporting).

## F. Biosecurity audits

Biosecurity protocols as specified in the Maine Aquaculture Association's Bay Area Management Agreement are to be followed at all marine sites. Periodic assessments of existing biosecurity measures will be coordinated through the ISA Program Veterinarian. Audits of marine sites, fish processing plants, marine vessels, or other epidemiological components may be completed by trained and experienced personnel as assigned by the ISA Program Veterinarian.

## G. 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; cage/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. All surveillance documents, laboratory reports, and other documents as required will be forwarded to the ISA Program Veterinarian. A flow chart reflecting the reporting mechanism is provided in [Appendix E](#). 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 ISA Program Veterinarian and Maine's DMR any suspected or confirmed cases of ISA within 24 hours of learning of the positive test results

or diagnosis. Accredited veterinarians shall submit all diagnostic samples for ISA to an approved laboratory in accordance with instructions provided by the ISA Program Veterinarian or Area Veterinarian in Charge (AVIC) and shall complete any and all procedures as instructed by this program by the ISA Program Veterinarian or AVIC. Accredited veterinarians shall use either USDA APHIS Specimen Submission Form (VS form 10-4) or other approved form developed by the ISA Program Veterinarian.

The specimen submission form under this program is shown in [Appendix F](#). It must be filled in completely and must be signed and dated by the submitting accredited veterinarian.

**Approved Laboratories:** The approved laboratory shall report results of surveillance laboratory tests to the ISA Program Veterinarian and the accredited veterinarian within 2 weeks of receiving the samples submitted for ISA surveillance. The approved laboratory shall within 24 hours of test completion report to the ISA Program Veterinarian, the Commissioner of the Maine DMR, and to the accredited veterinarian all test results for any official test for ISA. If viral culture is required to resolve the results of ISA assays, the finalized report of viral culture results must be submitted to the ISA Program Veterinarian within 30 days from the day of sample receipt by a USDA APHIS-approved laboratory.

All surveillance documents and laboratory reports will be forwarded to the ISA Program Veterinarian. A flow chart reflecting the reporting mechanism is provided in [Appendix E](#).

**ISA Program Veterinarian:** The ISA Program Veterinarian is responsible for overall field management of the ISA program in Maine and reports directly to the Area Veterinarian in Charge (AVIC) for New England. The ISA Program Veterinarian may also consult with the USDA APHIS VS Regional Director, and headquarters staff as necessary to carry out this program. Major duties and responsibilities for this veterinarian include but are not limited to: assigning personnel as needed to carry out program goals; collating surveillance reports from industry, site veterinarians, approved laboratories; resolving the accuracy of the respective surveillance reports; determining ISA Program Site Categories; reviewing laboratory procedures; processing indemnity payment; and meeting with industry on program related issues. The ISA Program Veterinarian will, under conditions of confidentiality, have access to

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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 AVIC and for the generation of reports and information for utilization of the USDA APHIS ISA Technical Board in resolution of ISA incidents. The ISA 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 AVIC, 2) the Commissioner of DMR, and 3) members of the USDA APHIS ISA Technical Board.

## Part III. Standards for Disease Control Actions and Management

### A. ISA Program Categories

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

**Table 2: ISA Program Categories and Description**

<b>ISA Program Category</b>	<b>Category Description</b>
Category 1 (presumed negative)	ISAV has not been detected at a cage or site participating in active ISAV monthly surveillance testing; considered negative for ISA.
Category 2 (suspect)	ISAV has been detected by at least one diagnostic test in at least one fish; considered suspect needing further evaluation and testing within 7 days.
Category 3 (infected)	A pathogenic genotype of ISAV has been detected by at least two diagnostic tests in at least two fish from the same cage. For subsequent cages on an infected site, a cage may be deemed Category 3 based on two fish found positive by one test (PCR, IFAT, gross pathology) accompanied by a positive virus isolation from individual samples or single cage pools.
Category 4 (diseased)	As for Category 3 above, plus clinical disease is present (as diagnosed by a veterinarian).
Category 5 (diseased)	As for Category 4 above, plus mortality consistent with ISA is present at the average rate of 0.05% per cage population per day over one week.
Category 6	Cage or site previously classified as Category 2 through 5 has been harvested or fallowed.

Cages or sites for all companies participating in the ISA program will be categorized according to the surveillance test results criteria included in Table 2 above. Cages or sites will be categorized or re-categorized as often as necessary to reflect management actions taken under this section, or taken at the discretion of individual company policy, if that policy does not conflict with the intent of these Standards.

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

1. **ISA Program Site Category 1** cages or sites will continue to conduct surveillance at monthly intervals as detailed in [Part II B](#) (Surveillance Requirements).
2. **ISA Program Site Category 2** cages or sites will be required to undergo additional sampling within 7 days of the report of a positive test result to ascertain a more precise program category. Based on the particular situation for the site where a Category 2 cage may be found, at the discretion of the ISA Program Veterinarian: 1) the USDA APHIS VS AVIC may be notified; 2) the ISA Technical Board may be convened; 3) additional epidemiological information may be requested; or 4) consultation with USDA APHIS VS headquarters staff. Category 2 sites will revert to Category 1 after four negative biweekly veterinary surveillance inspections and in consultation with the Program Veterinarian.
3. **ISA Program Site Category 3, 4 or 5** cages will be ordered to be depopulated by the Maine DMR.
4. Alternatively or simultaneously, the Maine Commissioner of Marine Resources or his designate may put any site falling into ISA Program Category 2 through 5 under restriction or quarantine, if the Commissioner feels such action is necessary to prevent the spread of a disease or disease agent. For sites under restriction, no additional fish may be transferred from the site unless specifically authorized in writing from USDA APHIS, or DMR. Under conditions of quarantine, no additional fish may be transferred to the site, and no live or dead fish may be removed from the quarantined site unless specifically authorized in writing from USDA APHIS and DMR for purposes of slaughter or disposal. No equipment or boats are to leave a quarantined site unless authorized by the ISA Program Veterinarian or Maine's DMR. The site's authorized veterinarian will also be informed of any restriction or quarantine measures.
5. The ISA 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**

All ISAV-infected fish will be quarantined to the site and cannot be moved for any purpose until a properly executed permit VS form 1-27 ([Part III E](#)) is issued. Quarantines will be issued using 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.

## **C. Indemnity**

As stipulated in Title 9 CFR Part 53.2, and when funding is available from USDA APHIS, indemnity payments will be made to producers complying with ISA Program Standards for fish destroyed due to ISA. These may include ISAV infected as well as ISAV exposed fish. The ISA Program Veterinarian will make final determination as to the ISA Program Site Category of the site and approve indemnity payments to producers. A schedule of payments will be coordinated by the USDA APHIS Program Veterinarian.

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 USDA APHIS ISA 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 III F of these Standards

## **D. Depopulation**

All depopulation orders will arise from the regulatory authority of the Commissioner of the Maine DMR. Depopulation may be ordered based on cage or site ISA Program Category or as a result of consultation with the ISA Program Veterinarian or recommendations from the ISA Technical Board. If the AVIC, ISA Program Veterinarian and/or the DMR Commissioner do not agree with the recommendations of the ISA Technical Board, *any party* may elect to discuss the recommendations with the Board or its Chair if necessary, and request any of the alternatives listed under [Part II D](#) of these Standards.

The depopulation of any unit which a company is ordered to comply with must be complete within 10 days after a depopulation order is issued. 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 ISA Program Veterinarian before the original deadline has passed. Depopulation must be accomplished minimizing exposure of all other fish at the site and in bay waters and in accordance with Appendices [A](#) and [B](#) of this document. Standards for fish harvesting, transportation, and processing are included in Table 4 of [Appendix C](#) and in Appendices [A](#) and [B](#). Fish can be moved to composting sites, landfills, or to approved fish processing plants only by permit and must meet all applicable state regulatory criteria. All methods of harvest or transport for disposal shall comply with either the stipulations of these ISA Program Standards, Maine MRSA [Chapter 24.21](#) and/or any pertinent DMR Emergency regulations, or with the stipulations of the Maine Aquaculture Association (MAA) Finfish Bay Management Agreement currently in effect, providing that the latter does not conflict with or suborn the intentions of the former. ISA Program Site Category 4 or 5 fish 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 ISA Program

Veterinarian will oversee the depopulation procedures for as many units as may be practical, or may delegate other representatives to do so.

## **E. Permitting**

All fish, nets, equipment, and other fomites capable of transmitting ISA must be permitted for removal from all [Category 2](#) and higher sites. Permits (VS form 1-27) will be issued by USDA APHIS to allow movement of fish to approved fish processing plants, fish composting, rendering or landfill sites.

## **F. Cleaning and Disinfection**

The level, specification and schedule for cleaning and disinfection will follow guidelines established in the MAA Finfish Bay Management Agreement (see [Appendix B](#)). The ISA Technical Board may be convened to review issues surrounding cleaning and disinfection and make recommendations to the ISA Program Veterinarian. Guidelines for cleaning and disinfection are indicated in **Table 3** below.

**Table 3: Cleaning and Disinfection Levels and Specifications:**

<b>SITE HEALTH STATUS</b>	<b>CLEANING &amp; DISINFECTION LEVEL</b>
---------------------------	--

No clinical disease of regulatory concern has occurred and no cages of fish were depopulated due to confirmed positive ISAV tests during the entire production cycle of the last year class to occupy the farm.	<b>1</b>
A clinical disease of regulatory concern or any cage of fish was depopulated due to confirmed positive ISAV tests.	<b>2</b>

### **Level 1:**

- Remove all fish being held on the lease site;
- Remove, clean and disinfect all nets according to the protocols specified in [Appendix B](#);
- Properly clean and disinfect all equipment including, but not limited to, cages, ropes, boats, grading, harvesting, and feeding equipment according to the protocols specified in [Appendix B](#);
- Minimize all traffic to and from the site; and
- Leave site fallow for as long as it is reasonably possible.

### **Level 2:**

- Perform all Level 1 protocols. In addition, perform the following protocols;
- Remove to land all cages, barges, boats, mooring balls and feeding pipes;
- Scrape, clean and if possible disinfect all mooring lines and grids; and
- When towing cages, barges, boats, mooring balls and feeding pipes to land, care should be taken to not transit close to any farms that have not triggered Level 2 fallowing requirements. If this is not possible, cages and all equipment should be scraped, cleaned, and disinfected in situ.

## **G. Fallowing**

For the purposes of this program fallowing is the emptying of a cage or in some cases an entire site of fish. Fallowing time is the interval between the time all fish are removed and when fish are restocked in the cage or site. Fallowing time period begins only upon completion of all cleaning and disinfection procedures of cages, pens, equipment, and other fomites as required.

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The minimum fallowing time for infected cages or sites is dependent upon average water temperatures and is described in [Appendix C](#).

## **Part IV. Administrative procedures**

### **A. Supervision of the ISA Program**

The USDA APHIS VS, [Area Veterinarian in Charge](#) (AVIC), located in the New England Area office in Sutton, MA, has administrative and supervisory responsibility for administering and managing all USDA, APHIS, VS programs in New England including the ISA Program in Maine. The AVIC will appoint a veterinarian to be dedicated full-time to the ISA Program, designated as the ISA Program Veterinarian.

The USDA APHIS VS [ISA Program Veterinarian](#), located in Maine, is responsible for management and administration of the ISA Program. Major duties and responsibilities include coordinating and implementing all aspects of the ISA Program in Maine, and reports directly to the USDA APHIS VS AVIC. The ISA Program Veterinarian will operate from an administrative base to be located in Maine in the general vicinity of northeastern coastal salmon aquaculture operations. The ISA Program Veterinarian will also facilitate communications with contact personnel at Maine DMR, Maine Department of Inland Fish and Wildlife, Maine Aquaculture Association, USDA APHIS approved diagnostic laboratories, APHIS VS aquaculture staff in Riverdale, MD, the media, or other necessary parties. Unless otherwise provided, all communications from the ISA Program Veterinarian will take place under conditions of confidentiality to the fullest extent of federal law. The ISA Program Veterinarian will develop and coordinate the dissemination of all necessary USDA APHIS VS forms specific to the ISA Program, and develop, supervise, coordinate and otherwise implement the provisions of the ISA Program contained in this document. The ISA Program Veterinarian will serve as liaison between USDA APHIS VS and any other parties for all actions to be undertaken involving the detection or elimination of ISAV-infected or ISA-diseased fish under this program.

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

## **B. Access to sites**

USDA APHIS VS personnel shall have access to all sites necessary to carry out any and all aspects of this ISA program during normal hours of operations.

## **C. ISA Technical Board**

The ISA Technical Board provides and offers recommendations to USDA APHIS VS about implementing aspects of the ISA Program. Its purpose is to provide broad, balanced, and scientifically sound input to the ISA Program Veterinarian. The Technical Board can provide input as requested to aid in the resolution of equivocal or conflicting laboratory results, aid in the evaluation of site categorization, and may periodically review the ISA Program recommending program improvements and enhancements.

## **D. Confidentiality**

Confidentiality will be maintained to protect proprietary information submitted by the participants in the program. The agency will 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 specific indemnity amounts will not be released. Additionally, forms used to submit samples to the laboratory will not contain a site identifier. The appropriate accredited veterinarian will receive copies of the lab submission forms with the site identifiers.

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# **APPENDIX A: Fish Health and Biosecurity Guidelines (taken from the Maine Aquaculture Association Finfish Bay Management Agreement January 2002 Appendix D)**

In order to minimize disease risks and maximize fish stock performance and animal welfare, all operators should continuously strive to improve their husbandry and farm management practices. All Bay Management Groups shall develop and implement a Bay Management Area Fish Health and Biosecurity Plan.

These Guidelines are recommended for immediate implementation of ALL marine sites in Maine and are intended to reduce the risk of the introduction and spread of infectious diseases (such as ISA) due to human activities onto and between marine sites and cages by movement of farmed fish, equipment and people.

## **1. Biosecurity Audits**

- All fish culture and salmonid processing facilities should be subject to third party biosecurity audits at least once a year.
- Facilities that are confirmed positive for ISA or process fish from confirmed positive ISA sites should be subject to biosecurity audits more frequently.
- Facilities that exhibit consistently poor performance on biosecurity audits should be subject to more frequent biosecurity audits.

## **2. 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 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.”

## **3. Broodstock**

When healthy broodfish are being stripped, the following guidelines should be followed:

Wherever possible, broodstock should be stocked, raised, and/or held only on specially designated broodstock sites. These sites should not hold fish other than broodstock candidates or selected broodstock. Broodstock should not be reconditioned and/or moved to production sites for growing to harvest.

Movement of broodstock candidates to marine broodstock sites from marine sites in other bay management areas is prohibited. Movement of broodstock candidates to marine broodstock sites from other marine sites within the same bay management area is strongly discouraged. Any movement of broodstock or broodstock candidates is only allowed according to the guidelines outlined below under bullets three and four in the “Fish” section.

- Movement of selected broodstock to freshwater sites is permitted only from marine sites participating fully in the industry fish health surveillance program and according to the guidelines outlined in the previous paragraph.
- Eggs and juvenile stages at fresh water facilities must never share the same facility area or water mass with broodstock moved from marine sites.

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- Movement of broodstock from a marine site to a fresh water facility without the appropriate fish health testing and inspections as required by state regulations may jeopardize the fresh water facility's "qualified source" status.

#### **4. Fish**

The following guidelines will govern fish stocking and movement:

- Adopt a single year class per site stocking policy.
- Minimize movement of fish between marine sites.
- Never move fish between ISA-suspect and ISA-positive marine sites and sites with unknown disease status.

#### **5. Dead fish collection, storage, and disposal**

The following standards are indicated:

- No dead or moribund fish should be released into the water.
- Collect all dead fish at least once weekly. Aim at daily collection.
- Use separate mort bags for each cage. Alternatively disinfect nets between cages by submersing net for 15 minutes in 100ppm iodine disinfectant.
- Dip and remove moribund fish from cages. Clean and disinfect nets used to dip moribund fish between cages.
- Remove dead fish from site as soon as possible.
- Use only mort totes in good condition, never cracked and leaky ones.
- Cover mort totes with properly fitting lids.
- Store mort totes away from feed.
- Place a footbath with disinfectant in the immediate vicinity of the mort tote.
- Empty mort totes as soon as possible, preferably after each mort dive.
- Disinfect area beneath and surrounding mort tote whenever it is removed for disposal of dead fish.
- Disinfect cage handrails and net areas above the water line that mort bags come in contact with.
- Use Company and site-specific equipment for storage and transport of dead fish. Mark or color code containers with company ID and site ID.
- Store dead fish on shore in plastic lined xactics specifically marked for mortalities.
- Dispose of dead and moribund fish by taking them off the site as soon as possible in leak-proof and covered totes or xactics and to an approved disposal site.
- Clean and sanitize mort containers before returning to the designated site. This is best done immediately after disposal.
- For proper cleaning and disinfection of containers designed for dead fish refer to Appendices F and G of Maine Aquaculture Association's Bay Area Management Agreement.

#### **6. Blood water:**

Exposure to blood water from ISAV infected fish is considered a high risk factor. The following minimum standards should be observed:

- Live haul to the processing plant, and no slaughter on site is strongly recommended.
- If live haul is currently not practiced, then blood water shall not be released into the marine environment without proper treatment.
- All attempts should be made to prevent leakage and spills during harvest and transport.
- For disposal of and disinfection of blood water, refer to Appendices F and G of Maine Aquaculture Association's Bay Area Management Agreement.

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## **7. Divers and diving gear**

- Properly disinfect diving gear before first cage, between cages, and after last cage.
- Dive cages with elevated mortality last.
- Dive the youngest fish first.
- All diver equipment shall be site-specific.
- If a diver must dive more than one site using the same gear, it is imperative that all gear is disinfected between sites. Specifically, gear should be disinfected after the last cage at the first site and allowed to AIR DRY. At the second site, the gear should be disinfected prior to diving the first cage.
- Diver attendants shall wear designed site-specific rain gear and boots. This gear must be properly cleaned and disinfected after each use.
- The dive boat should be site-specific.
- Diver attendants should not handle feed on that day. If dive attendants must handle feed, they must comply with all proper disinfection procedures. Ideally, the site should have a separate feed and diving crew.

## **8. Personnel**

- Limit traffic to sites and require that EVERYONE going to the site properly disinfects on the wharf or boat, and that EVERYONE leaving the site properly disinfects on the boat.
- 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 (i.e. arms, hands) soon afterwards.
- Keep employees, including divers, site-specific. Proper disinfection and air-drying of all gear must be enforced between sites when this is not possible.
- All people traveling to a site must wear footgear that can be disinfected by stepping into a footbath. Sneakers are not acceptable.

## **9. Equipment**

- Properly clean and disinfect all equipment after each use. This includes mort totes, diving gear, mort bags, harvest and grading equipment, xactics used to transport harvested fish, dip nets, etc.
- Do not use cracked, leaky totes/xactics.
- Retire wooden equipment, including wooden pallets, boats and barges, because they cannot be properly disinfected.
- Properly disinfect all nets prior to their re-use. Ideally, nets should be washed on shore and all water used for cleaning be collected and disinfected before its disposal. If nets must be washed in the water, then they should be washed on site, rather than a central location, to prevent the potential for transmitting pathogens contained within the debris on the nets from one site to another.
- Nets from confirmed positive ISA sites should never be washed on site. They should be taken to land and handled under the guidelines established in [Appendix B](#).
- Properly disinfect everything that comes in contact with dead, moribund, sick, or processed fish or fish parts, and blood water.
- Prohibit sharing equipment between sites. This includes mort totes, mort bags, nets, or diving gear, etc. When it is not possible to keep equipment site-specific, ensure that it is properly cleaned and disinfected before leaving one site and that it is properly disinfected at the new site before it is used on that site.
- Nets, totes, and gloves used during sea lice monitoring shall be disinfected between cages.

## **10. Boat traffic**

- All boats should be subject to annual third party biosecurity audits.
- Boats operating around confirmed positive ISA sites should have more frequent biosecurity audits.

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- All boats must have a clearly established “home” bay management area they operate out of. Boat traffic between marine sites and between processing plant wharfs should be minimized.
- Wherever possible, boat traffic between bay management areas should be eliminated.
- When boat traffic between marine sites or between marine sites and processing plant wharfs is unavoidable, boats should be disinfected as per the protocols and levels specified in [Appendix B](#).
- Where boat traffic between bay management areas is unavoidable, boats should be disinfected as per the protocols and levels specified in [Appendix B](#).
- No “Bus Stop” traffic patterns are allowed unless it is the sequential delivery of fish, goods, or services to sites that have no fish on them and have been appropriately disinfected and fallowed.
- This does not preclude delivery to a site containing fish as long as the boat does not subsequently proceed to another site.
- Where a boat travels into or operates in a bay management area other than its “home” bay management area, it should operate according to the standards established under the Bay Management Plan of the bay management area it is operating in.

# **APPENDIX B: Disinfection Guidelines (taken from the Maine Aquaculture Association Finfish Bay Management Agreement January 2002 Appendix G/Cobscook Bay, Bay Management Plan, Section 14, October 24, 2002)**

All Farm Occupants in a bay management area must develop site-specific disinfection programs. Site-specific disinfection programs must address all three phases involved in disinfection procedures; cleaning, disinfection and isolation;

- Wherever possible, site-specific disinfection programs must address all current known transmission and infection risks.
- Any disinfection procedure can be rendered ineffective by poor quality control or implementation. All site-specific disinfection programs must include components that demonstrate that Farm Occupants and management continuously strive to ensure all employees recognize the importance of proper disinfection procedures.
- Site-specific disinfection programs must include adequate documentation components in order to verify consistent implementation and identify employees responsible for their implementation;
- All disinfection procedures should only use cleaning agents and disinfectants approved for use by the EPA and USDA.
- Disinfection procedures should not include any off label use of cleaning agents and disinfectants.
- All disinfection procedures should be consistent with manufacturers' recommendations with respect to worker health and safety.
- All disinfection procedures should comply with EPA and DEP regulations pertaining to the discharge into the environment of the cleaning agents and disinfectants.
- Site-specific disinfection programs must include procedures that assure that subcontractors used by Farm Occupants understand and follow disinfection guidelines outlined in this Appendix and all other relevant protocols in the Bay Management Area Plan.
- All site-specific disinfection programs and procedures should be consistent with the guidelines established by the Maine Aquaculture Association Fish Health Committee. These Guidelines are as follows:

## **Effective Disinfectants**

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

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

- sodium hypochlorite (100-1,000mg/l water for minimum of 10 minutes);
- iodophor (100-250mg/l for 10 minutes);
- 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/cm<sup>2</sup>) ; and
- Sodium thiosulfate can be used to neutralize chlorine or iodine disinfectants
- Virkon<sup>®</sup> S (2% solution/10 minutes; followed by water rinse)

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Note: The choice of a particular disinfectant should be based on its efficacy in a particular application, whether it is approved by EPA and USDA for that application, and what, if any, environmental or worker safety risks may be associated with its use.

## **Egg Disinfection**

- Contamination of gametes with urine, feces, blood or other organic matter should be avoided during spawning.
- Fertilized eggs should be rinsed thoroughly with fresh water.
- Disinfection of pre-hardened eggs should occur as soon after fertilization as possible, using a buffered iodophor at a concentration of 100ppm for 10 minutes.
- Great care must be taken to separate pre-disinfection activities (dirty area) from fertilized disinfected eggs (clean area). No equipment or personnel should be allowed to cross these areas.
- Disinfection of eyed eggs should be conducted using iodophor solution to give 100ppm prior to hatch or movement to another location.

## **Equipment**

To achieve maximum efficacy of disinfectant, all objects prior to disinfection must be thoroughly cleaned and free of all organic material.

- Remove debris and organic fouling with brush and/or high pressure hose.
- Clean equipment using a detergent prior to disinfecting.
- All other equipment used in the cages must be cleaned and disinfected before being used in another cage.
- Use separate equipment for separate sites.

## **Visitors**

- Limit visitors to your site.
- All visitors should be issued appropriate footwear that is site specific and easily disinfected. Sneakers are not appropriate.
- Disinfect boots and gear on all visitors before entering and leaving your site and/or vessels. Sites should have gear designated solely for visitors rather than allowing visitors on site with their own gear.
- Footbaths must be present throughout the site and replaced daily.
- Staff gear should only be used at one site and regularly washed and disinfected, particularly after harvesting.

## **Nets**

- Nets must be cleaned of all organic material before disinfecting.
- Nets should be cleaned on land, if possible.
- All nets from confirmed positive ISA sites should be taken to land for cleaning and disinfection.
- Dirty nets being transported to shore should be contained in a manner, which prevents the loss or spillage of organic matter.
- Nets, which cannot be cleaned on land, should be cleaned as far away from any cages, but still on site, if possible.
- Nets from a positive cage should be taken ashore to be cleaned and disinfected at a proper facility. All efforts should be made to contain all material from the net (mussels, etc.) from being spread around the wharf, loading areas, etc.
- Material collected during net cleaning should be disposed of appropriately.
- Care should be taken to ensure that the cleaning/disinfecting procedure used for nets does not adversely affect the anti-foulant treatment or breaking strength.

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## Diving

- If possible, a separate boat or scow (steel or fiberglass construction) should be dedicated to diving only.
- Disinfect divers thoroughly between cages, before, and after leaving your site.
- Dive on suspect and/or positive cages last and disinfect between diving on cages by full immersion.
- Diver must have a complete, separate suit for each farm.
- Mort tubs, disinfectant tubs for diver, etc. can be kept separate and away from remaining equipment. Proper disinfection practices should be followed, i.e., disinfect boat, tubs, raingear, etc. after every dive.
- All mortalities are to be taken ashore and disposed of in an approved facility. Use totes for morts and morts only. No cracked or leaky totes should be used. Use plastic liner for mort totes to prevent spillage during transport. Mort containers should be site specific and have the site name clearly identified.
- Mortalities should be removed at a minimum weekly; however, the goal should be daily removal. Have diagnostics done monthly on moribund fish. Have separate mort bags for each cage or alternate bags between cages so that one bag will soak in disinfectant for 10 minutes or more. Mortalities should be placed in a leak-proof box with a liner.
- Mortalities are to be removed daily if they exceed 0.05% per day. Each pen must have its own mort bag and any weak fish should be dipped from the surface.
- Personnel tending divers should have gear designated solely for this purpose.

## Wharves

- At the wharves, 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.
- Always load/unload the boom truck on even ground. Strap harvest tanks evenly.
- All trucks must be equipped with disinfectant, a sprayer, and a brush.
- Disinfect barge/boat and all gear after leaving the wharf.
- Disinfect boom truck and all gear including straps after loading/unloading the boat/barge.

## Vessels

- All boats should maintain a disinfection log that documents the cleaning and disinfection procedures used on the boat.
- 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 boat were cleaned and disinfected; the cleaning, and disinfectant agents used, the date of such procedures, and the signature of the responsible employee or boat skipper.
- All boats shall have their hulls cleaned and scraped regularly to minimize bio-fouling.
- Boats operating in bay management areas with positive ISA sites must have their hulls cleaned and scraped twice annually or once annually and use an effective anti-foulant hull paint.
- All boats carrying live or dead fish must clean and disinfect all areas of the boat from the waterline up before and after each trip.
- Particular attention should be paid to areas of the boat and fish handling equipment that come in direct contact with fish or water fish have been in.
- All boats carrying live or dead fish must fill out their disinfection log after each day they have carried fish.
- When cleaning and disinfecting any boat, particular attention should be paid to areas that are difficult to access. Wherever possible, vessel and equipment design should minimize such areas.

Two levels of boat disinfection are to be used. The level of disinfection required is determined by the operational circumstances as defined in the Table below.

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<b>OPERATIONAL CIRCUMSTANCES</b>	<b>DISINFECTION LEVEL</b>
Travel from confirmed/suspected ISA site to ISA negative site	2
Travel from confirmed/suspected ISA site to another confirmed/suspected ISA site	1
Travel from ISA negative site to a confirmed/suspected ISA site	1
Travel from a bay management area with any confirmed/suspected ISA positive sites to a bay management area with no confirmed/suspected ISA sites	2
Travel from a bay management area with any confirmed/suspected ISA positive sites to a bay management area with any ISA negative sites	2
Travel from a bay management area with all ISA negative sites to a bay management area with all ISA negative sites	1
Travel from a wharf associated with loading/off loading of vessels coming from confirmed/suspect ISA sites to ISA negative sites or bay management areas with no confirmed or suspected sites	2
Travel from a wharf associated with loading/offloading of vessels coming from a bay management area with any ISA confirmed/suspect ISA sites to a bay management area with any ISA negative sites	2

**Level 1 Cleaning and Disinfection:**

- 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 anti-biological detergent and hot water; allow appropriate contact time.
- A low pressure applicator may be used to apply detergents.

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- A high pressure washer may only be used for initial cleaning prior to detergent application or after detergent application for rinsing detergent off after the appropriate contact time.
- After rinsing all surfaces, apply the appropriate disinfectant and allow the appropriate contact time.
- Do not rinse disinfectant off surfaces while still moored to the cage site or off loading/loading dock as rinsing with water from immediate proximity may re-contaminate the vessel.
- Do not rinse disinfectant off surfaces while in the immediate vicinity of any other farm site or dock during transit.
- Fill out and sign cleaning and disinfection log immediately.

**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 cleaning agents and disinfectants are repeatedly cycled through all pumps, pipes, hoses and/or valves that may have contacted fish, fish water or blood water.
- Slip or careen the vessel, clean, scrape, wash, and disinfect the hull.

## **APPENDIX C: Integrated Pest Management Guidelines (taken from the Maine Aquaculture Association Finfish Bay Management Agreement January 2002 Appendix E)**

All Bay Management Groups shall develop and implement a bay management area integrated pest management plan. IPM procedures include Best Management Practices that will reduce the need for use of chemicals or medications. Details of these procedures may be determined and recommended by the Industry Fish Health Committee. Bay Management Area IPM Plans should at a minimum include:

- Coordinated bay-wide treatment to reduce initial infection from external sources.
- Stocking of sites with single year class.
- Fallowing between year classes as per Table 4, and according to the standards and protocols outlined in Appendix D (of the Bay Management Agreement)
- Continuous efforts to minimize stress.
- Selection of fish strains for greater resistance to sea lice infection
- Use of late fall/early winter treatments to clean up fish.
- Use of filters/screens on water drain when using fish pumps.
- Use of cleaner fish (such as Wrasse) where available.
- Routine monitoring of sea lice populations at least bi-weekly, when water temperatures are greater than 8°C and monthly when water temperatures are between 4°C and 8°C.
- The maximum treatment threshold for sea lice counts is referenced in the appropriate INAD protocols. At the discretion of the licensed veterinarians, treatment may be initiated before such a high count is reached\*.
- All cages on a site should be treated simultaneously or within the shortest possible time.
- All Farm Occupants support the continued development of alternative therapeutant and management methods in order to reduce the development of possible resistance and minimize chemical use.
- In the event that carry over of market fish is used as a transition mechanism, all carry over fish must be treated for sea lice before stocking of different year class smolts at the same site.
- All fish treated for sea lice must not be harvested prior to the FDA mandated withdraw time.

**Table 4: Fallowing between year classes**

TEMPERATURE (°C)	MINIMUM NUMBER OF FALLOWING DAYS
> 11°	30
9-11°	35
<9°	42

\* Amended by the ISA Technical Board in 2007 & 2010 due to changes in therapeutant use between the time of the Bay Management Agreement writing and the date of this version of the Program Standards.

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## APPENDIX D: ISA Program Contacts

### USDA APHIS VS Area Veterinarian –in-Charge:

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USDA APHIS VS  
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Sutton Square Plaza, Suite 20  
Sutton, MA 01590  
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### ISA Program Veterinarian:

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USDA APHIS VS  
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August, ME 04333  
Ph: 207-287-7632 Fax: 207-622-6848 Cell: 207-837-2518  
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### Maine Department of Marine Resources:

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### Maine Department of Agriculture, Food and Rural Resources:

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### Maine Department of Inland Fish & Wildlife:

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### Micro Technologies, Inc.

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**Veterinary Contacts by Company**

Cooke Aquaculture (Phoenix Salmon; LR Enterprises; Cobscook Bay Salmon; Atlantic Salmon of Maine; Treats Island Fisheries)

Leighanne Hawkins, DVM  
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**Salmon Producers – Maine Contacts**

Cooke Aquaculture (Atlantic Salmon of Maine)

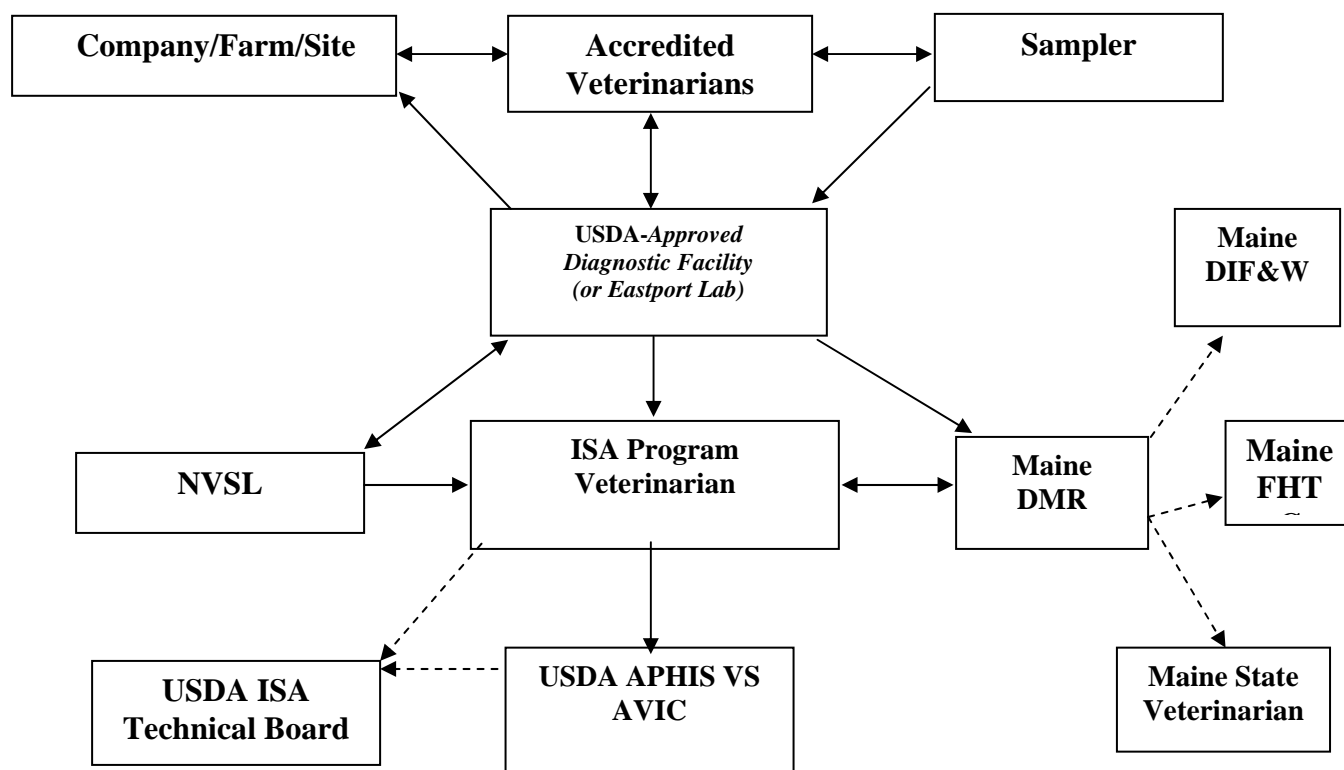
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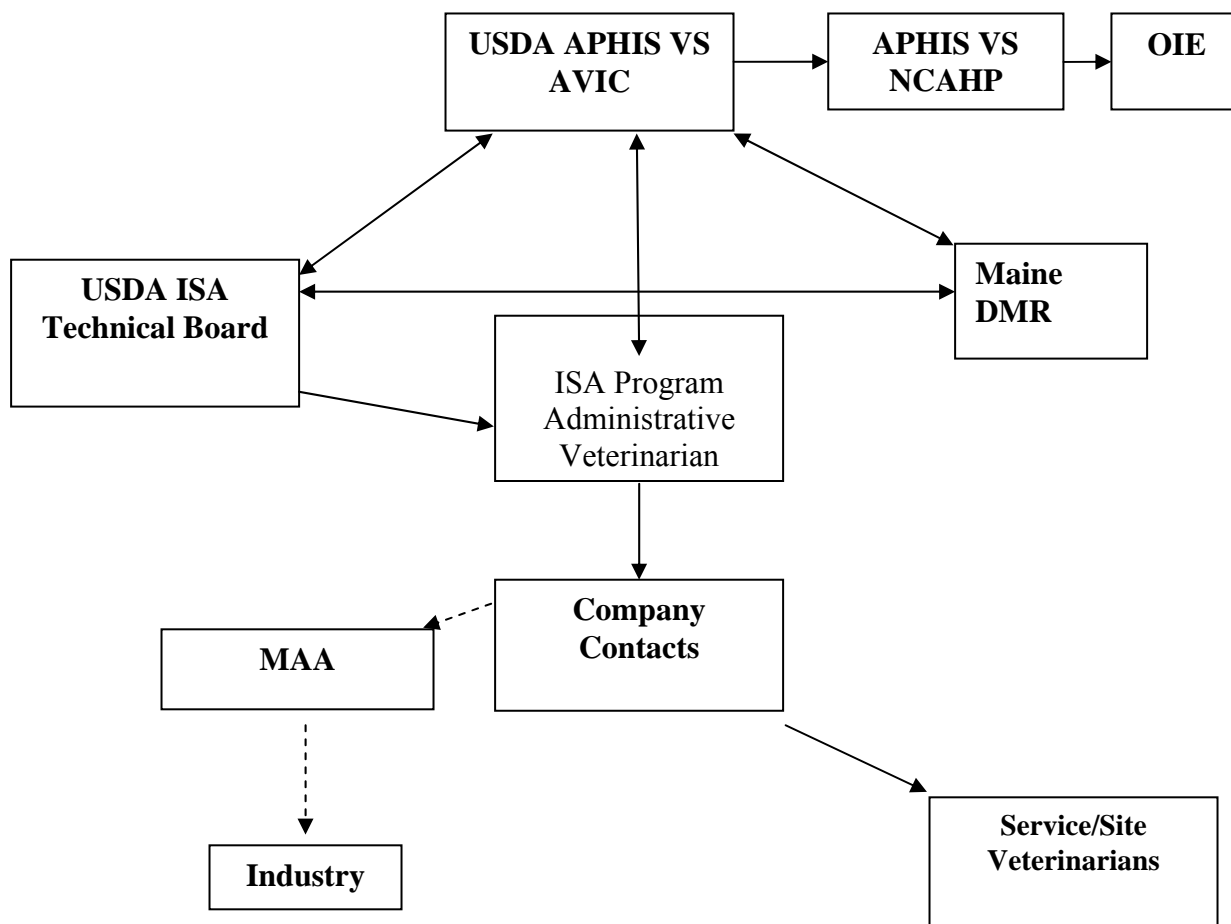
## APPENDIX E: USDA APHIS ISA Program Flow Charts

### Sample Collection, Submission and Testing Results Notification Flow



←----- = optional chain of reporting

## USDA APHIS ISA PROGRAM Action Notification Reporting Flow Chart



←----- = optional chain of reporting



# APPENDIX F: USDA Sample Submission Form

## USDA APHIS ISA 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 Program Accession #:** \_\_\_\_\_

**Laboratory Accession #:** \_\_\_\_\_

Tag # (opt.)	Fish #	Cage #	Viro Pool	Year Class	Date sampled	Water Temp °C	Sea lice counts	Date submitted	Lab Use Only		
									P CR	IF AT	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 Program Administrative Veterinarian      ☐ FedEx

Send report to (print name): \_\_\_\_\_, DVM  
 Supervising USDA APHIS VS Accredited Veterinarian \_\_\_\_\_  
 ISA Program Administrative Veterinarian Countersignature \_\_\_\_\_  
 Date \_\_\_\_\_

Maine DMR Site Identifier Code \_\_\_\_\_

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<sup>2</sup> Hold Ctrl and left click hyperlinks throughout the document to get to specific hyperlinked sections. In most cases, Shift + F5 will bring you back to the last three cursor locations.