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Fish Health Protection Regulations

# MANUAL OF COMPLIANCE

Ottawa 1984 (revised 2004)

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Canada

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## ABSTRACT

This revised manual explains the application of the Fish Health Protection Regulations under the Fisheries Act of Canada. It outlines the administrative and inspection procedures to be followed and provides step-by-step procedures for handling fish samples to test for specified bacterial, viral, and myxosporean pathogens of salmonids.

All movements of fish into Canada or between provinces require a permit. This permit may be issued only to producers who have a FHPR Fish Health Certificate that shows that their facility has been inspected according to this Manual of Compliance and indicating the FHPR pathogen profile for the facility.

The sampling procedures are based on the probability of detecting an infected specimen in a lot, assuming a certain prevalence of detectable infection. Selection, transportation, and laboratory handling of the samples are described in detail.

The methods provide for the detection of the following pathogens: the redmouth bacterium (*Yersinia ruckeri*); the furunculosis bacterium (*Aeromonas salmonicida*): the protozoans causing whirling disease (*Myxobous cerebralis*) and ceratomyxosis (*Ceratomyxa shasta*); the viruses causing viral hemorrhagic septicaemia, infectious hematopoietic necrosis, and infectious pancreatic necrosis; and other pathogens considered to be notifiable, such as the bacterial kidney disease bacterium (*Renibacterium salmoninarum*).

## I. INTRODUCTION

The future of Canadian fish culture and of recreational and commercial fisheries depends upon healthy fish stocks. International and interprovincial exchange of cultivated species continues to increase facilitating the dissemination of serious infectious diseases. Effective programs of prevention and control are necessary to prevent spread of fish disease agents and to alleviate this serious impediment to development of these fisheries. This revised Manual of Compliance explains the application of the Fish Health Protection Regulations under the Fisheries Act of Canada. It presents guidelines for producers, defines the roles of Fish Health Officials and Local Fish Health Officers, and outlines the sampling, handling, and diagnostic procedures that constitute inspections leading to the issuance of a Fish Health Certificate.

The Fish Health Protection Regulations, as published in the Canada Gazette, appear in Section II. They apply to all fish species belonging to the family Salmonidae of the genera listed in Schedule I. The Regulations are designed to minimize the risk of the spread of infectious diseases through inspection of wild and cultured fish stocks and to control the movement of infected fish into Canada and/or between provinces/territories. They apply to live and uneviscerated dead cultured fish, eggs (including fertilized eggs or gametes) of cultured and wild fish and products of dead, uneviscerated cultured fish destined to move into Canada or across provincial boundaries within Canada. In the event of violation of these Regulations, seizure and other powers of the Fisheries Act apply.

Compliance with these Regulations and the issuance of a Fish Health Certificate requires that an existing facility with eggs and/or fish of unknown FHPR pathogen status must have four satisfactory inspections over a period of not less than 18 months before a FHPR Fish Health Certificate may be issued. Inspections must be conducted at intervals of not less than 90 days and not more than 270 days. Aquaculture facilities wishing to export disinfected eggs only, need only be tested for viral agents. If eggs or fish are transferred from another source, the Fish Health Certificate for the receiving facility will be changed to reflect the FHPR status of the source (if disinfected eggs are transferred; only the status of the Schedule II viruses will be changed for the receiving facility). The facility will have to re-commence the inspection schedule at inspection one and have four inspections over a minimum of 18 months, with inspections being not less than 90 days or more than 270 days apart, before a Fish Health Certificate can be re-issued.

A new facility using an isolated water supply free of all species of fish and starting with stocks from a source with a valid Fish Health Certificate can obtain a Fish Health Certificate after only one inspection. In this circumstance the Fish Health Certificate must reflect the disease agent profile of the source, plus the results of the one inspection at the receiving facility. For a production facility to retain a Fish Health Certificate, consecutive satisfactory inspections must be conducted twice yearly ideally at 6-month intervals with inspections being not less than 90 days or more than 270 days apart. Whenever possible, inspections should be made during the spring and fall periods, with the final inspection having been made not more than 270 days prior to date of entry of a shipment of fish into a province. For all inspections, procedures outlined in this manual are to be followed. Persons wishing to upgrade their facility's Fish Health Certificate, from positive to negative for a specific pathogen, will be required to implement a

program to eliminate the specific pathogen(s) and have four consecutive negative (for the specific pathogen(s)) inspections over a minimum period of 18 months with inspections being not less than 90 days or more than 270 days apart.

Zoning is being developed to identify the presence/absence of specific diseases in areas or regions of Canada. The identification of zones will protect disease-free areas during aquatic animal transfers into Canada, between provinces and ensure Canada is consistent with the OIE International Aquatic Animal Health Code and the World Trade Organisation (WTO) Sanitary and Phytosanitary (SPS) Agreement. The development of zoning for Canada will be based on aquatic animal health data and is the responsibility of the Aquatic Animal Health Office.

If further clarification on the interpretation of these regulations is required, contact the Aquatic Animal Health Office, National Registry of Aquatic Animal Health, Fisheries and Oceans Canada, Ottawa, Ontario K1A 0E6; nrfd@dfo-mpo.gc.ca.

## II. REGULATIONS RESPECTINGTHE PROTECTION OF HEALTH OF FISH

*This copy of the Fish Health Protection Regulations is provided for ease of use. For all purposes of interpreting and applying the law, users should consult the Regulations as amended, as registered by the Clerk of the Privy Council and published in Part II of the Canada Gazette.*

### Short Title

1. These regulations may be cited as the Fish Health Protection Regulations.

### Interpretation

2. In these Regulations,

"approved" means approved by the Minister (approuvé)
"certificate" means a certificate issued in accordance with Section 6 (certificat)
"cultured fish" means a fish listed in Schedule I that is propagated by man in a fish culture facility and includes the eggs of such fish; (poisson d'élevage);
"Fish Health Official" means a person approved to inspect fish and fish sources for the purposes of these Regulations; (inspecteur sanitaire des poisons);
"import" means to bring into any province of Canada from any other country or any other province of Canada; (importer);
"import permit" means a permit issued pursuant to Section 4; (licence d'importateur);
"Local Fish Health Officer" means a person approved as a local fish health officer in charge of the administration and enforcement of these Regulations (agent local de protection de la santé du poisson);
"Minister" means the Minister of Fisheries and Oceans for Canada; (Ministre)
"wild fish" means a fish listed in Schedule I, other than a fish propagated by man in a fish culture facility; (poisson sauvage).

### Prohibition

3. (1) Subject to subsection (2), no person shall import cultured fish or eggs of wild fish without an import permit
(2) Subsection (1) does not apply to eviscerated cultured fish.

### Permits

4. Subject to section 5, a Local Fish Health Officer for a province may issue, to a person who applies for one, an import permit that authorises the person to import cultured fish or the eggs of wild fish into that province.

5. No import permit shall be issued unless the person who applies for the permit has obtained a certificate; and
(a) the certificate indicates that no disease or disease agent listed in Schedules II to IV was detected; or

(b) the Local Fish Health Officer is satisfied that none of the detected diseases or disease agents indicated on the certificate will be harmful to the conservation and protection of fish in the province of importation.

## Certificates

6. A certificate required pursuant to section 5 is issued by a Fish Health Official and

(a) certifies that the source of the fish was inspected in the approved manner; and

(b) indicates which, if any, of the diseases or disease agents listed in Schedule II to IV were detected during the inspection or inspections, as the case may be.

## SCHEDULE I
## Fish

All species and hybrids derived from species of fish belonging to the family Salmonidae, including the following genera:

| | |
|---|---|
| Pacific salmon | *Oncorhynchus spp.* |
| Danube salmon and Taimens | *Hucho spp.* |
| Atlantic salmon | *Salmo spp.* |
| Trout | *Salmo spp.* |
| Char | *Salvelinus spp.* |
| Grayling | *Thymallus spp.* |
| Lenok | *Brachymystax spp.* |
| Inconnu | *Stenodus spp.* |
| Whitefish | *Coregonus spp.* |
| Whitefish | *Prosopium spp.* |
| Ayu | *Plecoglossus spp.* |

## SCHEDULE II
## Diseases or disease agents found in live fish or their source

1. Any filterable replicating agent capable of causing cytopathic effects in the cell lines of fish specified by the Minister including, but not limited to:

   a. Viral Hemorrhagic Septicemia (Egtved) (Egtved virus, VHS)
   b. Infectious Hematopoiestic Necrosis (IHNV)
   c. Infectious Pancreatic Necrosis (IPNV)

2. Whirling Disease (*Myxobolus cerebralis*)
3. Ceratomyxosis (*Ceratomyxa shasta*)
4. Furunculosis (Aeromonas salmonicida)
5. Enteric Redmouth Disease (*Yersinia ruckeri*)

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## SCHEDULE III
**Diseases or disease agents found in dead fish or their source**

1. Viral Hemorrhagic Septicemia (Egtved virus, VHSV)
2. Whirling Disease (*Myxobolus cerebralis*)

## SCHEDULE IV
**Diseases or disease agents found in live fish or their source**

1. Myxobacterial infections
2. Bacterial Kidney Disease (KD bacterium)
3. Motile Aeromonad Septicemia (motile *Aeromonas sp.)*
4. Pseudomonad Septicemia (*Pseudomonas spp.)*
5. Vibriosis (*Vibrio spp*.)

# MANUAL OF COMPLIANCE

## III. GUIDELINES FOR PRODUCERS

The Fish Health Protection Regulations (FHPR) apply to all facilities from which live and dead cultured fish, fertilised eggs and gametes of cultured and wild fish, and products of uneviscerated dead cultured fish of all species belonging to the family Salmonidae, as designated in Schedule I, which will be shipped into Canada or from one province/territory to another. "Facilities" include all sites used for the propagation or holding of eggs and/or fish. Persons wishing to transfer fish into Canada or between provinces/territories within Canada should become familiar with all of the regulations (federal, provincial and municipal) of the region into which they plan to import fish since requirements may exist in addition to the Fish Health Protection Regulations.

See Section A, Importation Of Eggs and Fish, below for more details on the importation of eggs and fish. An import permit is required for each shipment of fish or eggs designated above, except for interprovincial shipments of dead cultured fish within Canada. These import permits may be issued only for shipments from facilities with a valid Fish Health Certificate indicating that the facility has been inspected by an approved Fish Health Official and has satisfied the inspection requirements detailed in Section B below. A list of Fish Health Officials approved for the inspection of facilities may be obtained from the National Registry of Aquatic Animal Health, Fisheries and Oceans Canada, Ottawa.

Within Canada, anyone wishing to obtain a Fish Health Certificate for their facility must provide fish at their own expense for inspection. Fish species, other than those listed in Schedule I that are being reared at the facility, are also subject to sampling by the Fish Health Official. The Fish Health Official has flexibility in establishing time and frequency of sampling and selection of fish and must have access to records relating to introductions, losses, disease prevalence and treatments.

Although not specified in the Regulations, surface disinfection of eggs prior to shipment is strongly recommended unless otherwise advised. A suggested procedure for disinfection is given in Section XII of this manual.

A Fish Health Official may wish to alter inspection procedures and/or Fish Health Certificate issuance conditions when circumstances do not allow for a straightforward application of the regulations and/or this Manual of Compliance (e.g. specialized fish growing operations, research facilities, seasonal operations, etc.). In these cases, a prior ruling on these alterations must be obtained from the National Registry of Aquatic Animal Health.

A fish health diagnostic laboratory may be located in a different province from that in which an inspection is being conducted, requiring the transport of fish samples across provincial boundaries. Such samples should be handled so as to avoid any potential transfer of disease. All persons involved in fisheries research must conform to the requirements and intent of the Regulations and be cognizant of regional conditions.

### A. IMPORTATION OF EGGS AND FISH

Before shipment, an import permit must be obtained from the Local Fish Health Officer of the province/territory to which the shipment is going. Import permits must accompany all shipments of fish or eggs. Offices at which the Local Fish Health Officers can be reached are listed in Appendix 2.

To acquire an import permit, producers must provide a copy of their Fish Health Certificate (Appendix 4) with the EXPORTER'S DECLARATION portion completed and signed by the owner or manager of the source facility. The IMPORTING INFORMATION portion must also be completed and contain the signature and address of the importing recipient.

An import permit allows the transport of fish from the source facility directly to the receiving facility. The replenishing of water in a truck or other shipment container at any location other than another facility with a Fish Health Certificate of equal or better fish health status or an isolated water source free of all species of fish is the same as an introduction of fish from a source without a valid Fish Health Certificate and invalidates the import permit.

**Importation of Disinfected Eggs of Wild and Cultured Fish**

Sources of eggs must be inspected by a Fish Health Official (FHO) and a Fish Health Certificate issued noting the presence or absence of filterable replicating viral agents, including their strains/serotypes if required by the recipient province. Viral agents include, but are not limited to:

Viral hemorrhagic septicaemia virus (VHSV)
Infectious Hematopoietic Necrosis Virus (IHNV)
Infectious Pancreatic Necrosis Virus (IPNV)

The Local Fish Health Officer (LFHO) may, after reviewing the Fish Health Certificate issue an import permit if the import of such eggs will not result in the introduction of a viral agent or strain/serotype of a viral agent listed above and not already known to occur in the receiving province.

In the absence of fish health data indicating the presence of selected pathogens, the LFHOs may designate areas in a province as free of selected pathogens, even though the pathogens have been detected in other parts of the province. LFHOs may reject applications to import eggs from a source that tested positive for a selected pathogen into an area designated free of the specified pathogen. The eggs must be accompanied by an import permit issued by the LFHO in the receiving province.

Sources of disinfected eggs need only provide information on virus testing when applying for import permits. Eggs must be disinfected at the source and in the receiving facility. If eggs are not disinfected, information on all diseases or disease agents listed in Schedule II of the Regulations (i.e. including bacteria and parasites) must be provided.

**Importation of Live Cultured Fish**

Sources of live cultured fish must be inspected by a FHO and a Fish Health Certificate issued noting the presence or absence of diseases/disease agents listed in Schedule II of the FHPR including filterable replicating viral agents, including their strains/serotypes, if required by the receiving province/territory. Viral agents include, but are not limited to:

Viral Hemorrhagic Septicemia Virus (VHSV)
- Infectious Hemaotpoietic Necrosis Virus (IHNV)
- Infectious Pancreatic Necrosis Virus (IPNV)

Schedule II pathogens also include:

- *Aeromonas salmonicida*
- *Yersinia ruckeri*
- *Myxobolus cerebralis*
- *Ceratomyxa shasta*

The LFHO may, after reviewing the Fish Health Certificate, issue an import permit if the import of such fish will not result in the introduction of a disease agent or strain/serotype of a disease agent listed above not already known to occur in the receiving province/territory.

In the absence of fish health data indicating the presence of selected pathogens, LFHOs may designate areas in a province as free of selected pathogens, even though the pathogens have been detected in other parts of the province. LFHOs may reject applications to import cultured live fish from a source that has tested positive for a selected pathogen into an area designated free of the specified pathogen.

If the fish at the source are clinically ill with Schedule II disease agent(s), no fish should be transferred until the disease episode is determined by a veterinarian to be controlled. The fish must be accompanied by an import permit issued by the LFHO in the receiving province.

**Dead, Uneviscerated Cultured Fish**

Sources of dead, uneviscerated cultured fish must be inspected by a FHO and a Fish Health Certificate issued noting the presence or absence of disease agents listed in Schedule III, including:

- Viral Hemorrhagic Septicemia Virus (VHSV)
- *Myxobolus cerebralis*

The LFHO may, after reviewing the Fish Health Certificate, issue an import permit if the import of such dead, uneviscerated cultured fish will not result in the introduction of a disease agent or strain/serotype of a disease agent listed above and not already known to occur in the receiving province.

In the absence of fish health data indicating the presence of selected pathogens, LFHOs may designate areas in a province or territory as free of selected pathogens, even though the pathogens have been detected in other parts of the province/territory. LFHOs may reject an application to import dead, uneviscerated cultured fish from a source that tested positive for a selected pathogen into an area designated free of the specified pathogen.

## B. REQUIREMENTS TO OBTAIN A FISH HEALTH CERTIFICATE

### Aquaculture Facilities

An existing facility with eggs and/or fish of unknown pathogen status must have four inspections over a period of not less than 18 months before a Fish Health Certificate may be issued. Inspections must not be less than 90 days or more than 270 days apart. Aquaculture facilities wishing to export disinfected eggs only need to be tested for viral agents.

When eggs or fish are transferred from a source having a fish health status less than that of the receiving facility, the Fish Health Certificate for the receiving facility will be changed to reflect the health status of the source (if disinfected eggs are transferred; only the status of Schedule II viruses will be changed for the receiving facility). If the source facility does not have a valid FHPR Fish Health Certificate for the receiving facility will be invalidated. The facility will have to re-start the inspection schedule at inspection one and have four inspections over a minimum of 18 months, with inspections being not less than 90 days or more than 270 days apart before a Fish Health Certificate can be re-issued.

It is the responsibility of the owner of the receiving facility to notify the FHO about any change in status of their Fish Health Certificate. The FHO will provide an amended Fish Health Certificate, copied to the National Registry of Aquatic Animal Health. The expiry date on the amended certificate will be the same as that on the voided certificate.

A new production facility located on an isolated water supply free of all species of fish and starting with stocks from a source with a valid Fish Health Certificate, can obtain a Fish Health Certificate after only one inspection. The Fish Health Certificate must reflect the disease agent profile of the source facility plus the results of the one inspection at the receiving facility.

Once a facility has received a Fish Health Certificate, two annual inspections ideally at approximately 6 month intervals are required to maintain the Fish Health Certificate. Inspections must not be less than 90 days or longer than 270 days apart.

### Eggs of Wild Fish

Wild adult broodstock must have a record of two consecutive annual inspections in the past two years before a Fish Health Certificate may be issued. Where it is not possible to sample the same populations in consecutive years (e.g. Pacific salmon), broodstock from the same area of the river must be tested in the second year.

### Upgrading Fish Health Certificates

Facilities wishing to upgrade their Fish Health Certificate from positive to negative for a specific pathogen will be required to implement a programme to eliminate the specific pathogen(s) and have four consecutive negative (for specific pathogen(s)) inspections over a minimum period of 18 months. Inspections must not be less than 90 days or longer than 270 days apart.

## C. ADDITIONAL DISEASE TESTING

If there is information indicating that a new strain or serotype of a pathogen listed in Schedules II, III and IV could be introduced into a receiving province with a shipment of eggs or fish, the LFHO in a receiving province/territory may request additional testing in accordance with regional/provincial/territorial policies or regulations to determine the strain and/or nucleic acid profile for a pathogen detected at a source facility, if there is information indicating that a new strain or serotype of a pathogen listed in Schedules II, III and IV could be introduced to the receiving province with a shipment of eggs or fish.

If additional testing is required by the LFHO, then certain conditions for testing will apply. Additional testing must be coordinated and/or supervised by a FHO or LFHO. Tests conducted shall have a level of sensitivity and specificity comparable with other routine regulatory diagnostic test, and test procedures must be accessible to both public and private laboratories doing health certification inspections. Additional testing must be conducted by a credible diagnostician, in a timely fashion, and with due regard to provisions for continuity of evidence.

## D. APPEAL PROCEDURE

Where the LFHO does not issue an import permit under the Fish Health Protection Regulations, the LFHO must provide written reasons for rejecting an application. The applicant may request a review of a rejected application by applying to the Assistant Deputy Minister (ADM), Science Sector, Fisheries and Oceans Canada for a review of the decision. The appeal application should be in the form of a letter to the ADM addressed to the attention of the National Registry of Aquatic Animal Health and received within 30 days of receipt of the decision by the LFHO not to issue the import permit.

The application must include a copy of the original application for the import permit, a copy of the reason(s) given for not issuing the permit, and a copy of the reason(s) for requesting a review. Any additional information that the applicant wishes to submit in support of the application must be included with the review request. The applicant must demonstrate that the decision not to issue the import permit was inconsistent with the Regulations. The National Registry will coordinate the review process, establish a Review Board, participate as an advisor on the Review Board, and hold, assemble and forward documentation to the ADM.

The National Registry will forward a copy of the application to the appropriate DFO Regional Director General and responsible provincial agencies with a request that the Regional Director General and responsible provincial agencies submit written comments to the Review Board with respect to the application. They should respond within 21 calendar days of receiving the application from the National Registry.

The Review Board must be established within 5 calendar days of receipt of the application and will consist of the National Registry and three independent persons selected by the National Registry in consultation with the applicant and the responsible federal and provincial agencies. The role of the National Registry on the Review Board will be advisory. The National Registry will not vote on the recommendation(s) set out in the report prepared by the Review Board.

The role of the Review Board in the review process is advisory. The Review Board provides a report, including its recommendation(s) to the ADM. The ADM makes the final decision whether an import permit should be issued. The Review Board may request written submissions from anyone who, in the opinion of the Review Board, should be consulted.

The Review Board will prepare a written report to the ADM within 30 days of the Review Board being established. In exceptional circumstances, the Review Board may request the applicant for a reasonable extension of time to complete its report. The report will contain the recommendation(s) of the Review Board with respect to the application.

Subject to the Access to Information Act and the Privacy Act as amended from time to time, the Review Board will provide copies of the documentation to be forwarded to the ADM to the applicant. The documentation will include a copy of the Review Board's report, recommendation(s), the written submissions of the Regional Director General and the responsible provincial agencies, and other written submissions obtained by the Review Board as part of its review. The applicant will have 10 calendar days from receipt of the documentation to forward a written submission setting out comments. The submission should be in the form of a letter to the ADM addressed to the attention of the National Registry.

The National Registry will forward the documentation to the ADM for review and a final decision. The documentation will include the applicant's application for review (including any supporting documentation filed with the application), the review submissions of the Regional Director General and responsible provincial agencies and any other written submissions obtained by the Review Board in the course of its review, the Review Board's report and recommendation(s), and the applicant's written submission setting our comments.

The ADM will make the final decision on whether to issue an Import Permit and will provide the decision and the reasons(s) for the decision in writing within 30 calendar days. The decision will be forwarded to the applicant, copied to the Regional Director General, responsible provincial agencies and the LFHO through the National Registry. If a review relates to a shipment of eggs, any juvenile fish that may hatch from these eggs before the completion of the review procedure will be subject to live fish health requirements of the amended Fish Health Protection Regulations and other related regulations and policies.

Table 1. Summary of review procedure time periods for import permit appeals.

| ACTION | ALLOTTED TIME PERIOD | EXPECTED ELAPSED TIME AFTER LFHO SENDS REJECTION LETTER TO APPLICANT |
|---|---|---|
| LFHO sends letter to applicant rejecting application to import eggs or fish | - | Day zero |
| Applicant sends appeal application to National Registry | 30 days | Day 30 |
| National Registry establishes Review Board | 5 days | Day 35 |
| DFO Regional Director General and responsible provincial agencies submit comments on appeal application to National Registry | 21 days | |
| Review Board prepared report and recommendations for ADM | 30 days | Day 65 |
| Applicant provides comments to National Registry on Review Board's report and recommendations | 10 days | Day 75 |
| ADM provides written decision on appeal to applicant | 30 days | Day 105 |

## IV. ROLE OF FISH HEALTH OFFICIALS

The Fish Health Official (FHO) must be a qualified specialist in fish disease diagnosis, have access to a laboratory equipped to undertake the diagnostic procedures outlined in this manual, and have received approval from the Government of Canada (Appendix 3). Those seeking approval are to contact the National Registry of Aquatic Animal Health, providing a copy of their curriculum vitae, three specimens of their signature, and an outline of their laboratory capabilities. All applicants will be required to answer a knowledge questionnaire based on the Fish Health Protection Regulations and the Manual of Compliance, which will be reviewed by a panel of disease specialists. Applicants will be advised as to the outcome of their application. The FHO approval will be re-evaluated by the National Registry of Aquatic Animal Health every three years and, if appropriate, renewed for an additional 3 years.

FHOs should avoid and prevent situations that could give rise to a conflict of interest, or the appearance of a conflict of interest to ensure their impartiality and objectivity are maintained when inspecting facilities under the FHPR. This can protect FHOs from conflict of interest allegations and avoid situations of risk. Conflict of interests include situations where a FHO has a monetary or other economic investment or interest in the facility being inspected, or where the facility is owned or operated by the FHO, or by a member of the FHO's family. FHOs should not solicit or accept transfers of economic benefit, beyond the payment for services rendered, which could compromise the integrity of the FHO. When in doubt, FHOs should contact the National Registry.

Evidence that an approved Fish Health Official is not following the intent of the Regulations, i.e., to prevent the spread of infectious disease agents by careful inspection of production sources and control of infected stock movements, by wilfully disregarding either inspection requirements and/or results of inspections, can lead to temporary suspension of approval or permanent removal of approval.

The Fish Health Official conducts an inspection by visiting the production site, viewing all parts of the site, and carrying out the procedures outlined in Sections VI-XI of this manual. The FHO should obtain information from the owner or manager of the site on identification of stocks being inspected and become familiar with records of introductions, losses, disease prevalence and treatment of fish in the facility over the past two years, or from the date of initial entrance to the FHPR inspection program. If all requirements of the Regulations are met and if the inspection schedules outlined earlier have been adhered to, the Fish Health Official may issue a Fish Health Certificate (Appendix 4). A copy of the certificate must be distributed to the owner/manager, a copy of which will accompany each application for an import permit; the National Registry of Aquatic Animal Health and to the Fish Health Official.

If Fish Health Officials have diagnostic information which they have gathered from sampling outside of the scheduled FHPR inspections or documented diagnostic information from other reliable sources, this information is to be used in determining the facility's FHPR status along with that collected during scheduled FHPR inspections.

In addition, Fish Health Officials must complete a Fish Health Laboratory Report (Appendix 5) and send a copy of the Lab Report to the National Registry of Aquatic Animal Health, the owner/manager of the facility being inspected to provide evidence of the inspection, and retain a copy for their own files. Fish Health Laboratory Reports of facilities outside Canada must also be sent to the National Registry of Aquatic Animal Health.

If the invalidation of the Fish Health Certificate of a facility is necessary because of failure to meet FHPR requirements, then the following procedures are to be carried out by the Fish Health Official: a letter outlining the reason(s) for invalidation of the Certificate and the steps necessary to regain a Fish Health Certificate are to be sent to the owner/manager of the facility and the National Registry of Aquatic Animal Health.

## V. ROLE OF LOCAL FISH HEALTH OFFICERS

Local Fish Health Officers (LFHOs), located within each region of Canada, administer these Regulations for their province/territory. Their responsibilities include a review of Fish Health Certificates and data relevant to a fish or egg source in question, a shipment of fish or eggs in particular, and the health needs of their region. Subsequent to Section 5 of the Regulations, they may issue import permits to applicants to allow passage of acceptable shipments of live fish or eggs entering Canada or moving between provinces/territories within Canada and uneviscerated dead fish entering Canada at border points. An import permit must accompany each shipment. A standard import permit is not provided with this manual since provinces or regions have developed import permits in response to their individual needs.

The notifiable disease agents in Schedule IV are not listed in Schedules II or III, but are considered important by the Fish Health Official. In special cases, just cause may exist for the Local Fish Health Officer to prevent the import of these notifiable disease agents or other fish pathogens into an area.

Local Fish Health Officers are located at offices listed in Appendix 2 and the National Registry of Aquatic Animal Health can be contacted for current information for any specific province/territory/region.

## VI. SAMPLING PROCEDURES

### A. CULTURED FISH

#### Sampling by lot

Unless otherwise noted (see VI.B.1), during an inspection, fish are sampled by "lot." A lot is defined as fish of the same age that have always shared the same water supply and that have originated from a discrete spawning population. In situations where this lot definition cannot be applied, the Fish Health Official will use their own discretion in dividing the fish into lots.

#### Selecting the sample

The method of determining the actual number of fish to be sampled from any particular lot is based on obtaining a 95% probability of detecting an infected specimen in a lot with an assumed prevalence of detectable infection of 5 or 10% (Table 1 p. 12). It is important to note that certain disease agents when in the carrier state are very difficult to detect. The Fish Health Official and the producer must be aware of this possibility. Statistical probabilities as stated in Table 1 may not apply in such situations. The samples must be taken under the supervision of the Fish Health Official in a manner and at a time that tends to provide the greatest opportunity of detecting any disease agent present. When the sample is being withdrawn from a lot held in a single holding unit (e.g. tank, raceway, or pond), the sample must be selected to contain as many moribund and freshly dead specimens as are available. If the previously mentioned lot of fish is being held in more than one holding unit, the total number of specimen to be collected would be the same as before. However, the specimens must be taken from the individual units in numbers that reflect the proportion of the lot held in each of the units. Again the sample from any given unit must consist of as many moribund and freshly dead fish as are available. If additional fish are required to complete the sample, apparently healthy fish may then be collected, or the Fish Health Official may elect to return within a 30-day period to complete collection of the required number of fish.

The samples should not be taken during or immediately after therapeutic treatment. Complete background information for all samples must be obtained. This includes data relating to any recent use of chemotherapeutants, the health history of the facility and the lots from which the samples were taken.

In the event that overt disease signs are noted at the time of sampling, procedures for the detection of notifiable disease agents and other pathogens should be carried out in addition to the procedures for identification of disease agents listed in Schedule II.

When samples of fish or their tissues are processed in pools (rather than individually), as in the virological assays, care must be taken to process those from apparently healthy fish separately from those of freshly dead or moribund fish.

Table 1: Sample size required to detect one or more infected specimens in populations (lots) with an assumed minimum prevalence of detectable infection of 5 and 10%. Calculations are based on a 95% level of confidence. For intermediate population sizes, use the sample size for the next larger population listed (Ossiander and Wedemeyer 1973)

| | Number of fish to be sampled when assumed prevalence of detectable infection is: | |
|---|---|---|
| Population Size | 5% | 10% |
| 50 | 29 | 20 |
| 100 | 43 | 23 |
| 250 | 49 | 25 |
| 500 | 54 | 26 |
| 1,000 | 55 | 27 |
| 2,500 | 56 | 27 |
| 5,000 | 57 | 27 |
| 10,000 | 57 | 27 |
| 100,000 | 57 | 27 |
| Over 100,000 | 60 | 30 |

**Sample size for viruses**

a. Production fish (non-broodstock fish): the sample size to be employed is one that gives a 95% probability of detecting an infected specimen in a lot, assuming the minimum prevalence of detectable infection is 5%.

b. Broodstock (sexually mature fish held for reproductive purposes): the same sensitivity in detecting infected individuals must be aimed at. Sampling must be conducted once yearly at spawning time. In those species for which spawning is a terminal event, tissue samples must be collected from all fish involved up to a maximum of 60 fish. For those species that are repeat spawners, 10%[1] of all spawners used up to a maximum of 30 fish, must be subjected to lethal sampling. The balance of the samples required to achieve the rate that provides a 95% probability of detecting an infected fish in a lot, assuming the minimum prevalence of detectable infection is 5%, is to be made up of reproduction fluids. Ovarian fluid must account for as many as possible of the reproductive product samples collected.

[1] The lethal sampling of only 10% of all the spawners does not conform to the sampling rates set out in Table 1; it is intended to conserve small but valuable populations of spawners that may live to spawn again.

**Sample size for bacteria**

a. Production fish must be sampled for bacterial pathogens at a rate that provides a 95% probability of detecting an infected specimen in a lot assuming the minimum prevalence of detectable infection is 5%. For routine purposes, only fish averaging 4 cm or more in fork length need be bacteriologically sampled. Reliable bacteriological sampling of fish smaller than 4 cm in fork length is technically more difficult: such fish need be sampled only when unusual and unexplained mortality rates or disease signs are observed.

b. Broodstock must be sampled for bacteria in accordance with the sample size already outlined for the lethal sampling of broodstock for virus (see VI A.3b).

**Sample size for parasites**

a. Production fish must be sampled for *Myxobolus cerebralis* and *Ceratomyxa shasta* at a rate that provides a 95% probability that an infected fish will be detected in a lot assuming the minimum prevalence of detectable infection is 5%.

*M. cerebralis*: The fish must be at least 120 days of age for the test to be meaningful because the spores on which the diagnosis is based are slow to develop. In fish grown or held at temperature below $12^{o}$ C, spore formation may take 9-11 months. (Taylor et al, 1973)

Deleted: ,

*C. shasta*: Routine monitoring of apparently healthy fish for the presence of spores need be performed only with fish averaging at least 120 days of age. Younger fish need to be examined for *C. shasta* only when unusual and unexplained mortality rates or disease signs are observed.

b. Broodstock must be sampled in accordance with the sample size already outlined for the lethal sampling of broodstock for virus (see VI A.3b).

**Times of sampling and frequency**

a. Production fish: Sampling will be conducted at least twice yearly with sampling times and frequency depending on local conditions and the discretion of the Fish Health Official. Because the detection of *M. cerebralis* and certain viruses is best accomplished using fish of a certain age, spring and fall sampling periods (March-May and September-November) are recommended.

b. Broodstock: Sampling will be conducted once yearly at spawning time (see VI. A.3b).

## B. WILD FISH

### Sexually immature fish

All sexually immature fish taken from the wild must be sampled at a rate that will give a 95% probability of detecting an infected specimen in the total catch, assuming the minimum prevalence is 5%. This sampling rate will apply to the types of pathogens (viral, bacterial, and myxosporean) mentioned earlier. The Fish Health Official may use considerable discretion when determining an appropriate sample size for wild fish.

### Sexually mature fish

If wild spawners or their fertilized eggs are to be collected, seminal and ovarian fluids must be taken from all the fish involved, up to a maximum of 60 fish. In those species for which spawning is a terminal event, tissue samples must be collected from all fish involved (lethal sampling) up to a maximum of 60 fish. For those species that are repeat spawners, 10%[2] of all spawners used or collected, up to a maximum of 30 fish, must be subjected to lethal sampling. These sampling rates apply to the types of pathogens (viral, bacterial and myxosporean) mentioned earlier. Again, considerable discretion must be used by the Fish Health Official when certifying wild stocks.

## C. FERTILIZED EGGS AND GAMETES

The sampling of fertilized eggs or the gametes of fish cannot be relied on to detect disease agents listed in Schedule II. The threat to fish health posed by such eggs or gametes must, therefore, be determined by examining the disease status of the parent fish.

## D. NON-SALMONID SPECIES

Other species not belonging to the family Salmonidae occurring at the same facility as salmonid species are subject to sampling by the Fish Health Official for disease agents listed in Schedule II or III.

[2] See footnote 1.

## VII. TRANSPORTATION OF SAMPLES

Fish samples must be handled rapidly in such a way that degenerative changes do not render diagnosis unreliable or impossible. If samples cannot be brought to the laboratory alive, they should be stored on ice or refrigerated for no longer than 48 hours.

### A. LIVE FISH

Live fish should be transported in sealed plastic bags that have been partly filled with water and charged with oxygen. The bags should be labelled showing name of hatchery, date of inspection, lot identifier, and number of fish. The bags may then be placed with ice in insulated containers. Under these conditions anaesthetics are not usually required.

### B. DEAD FISH

Fish sampled should be placed in sealed plastic bags (dead or moribund fish should be kept separately from healthy fish), labelled as for live fish and packed in an insulated container with a layer of ice around each bag.

### C. REPRODUCTIVE FLUIDS

Seminal and ovarian fluid samples should be collected in sterile test tubes and shipped in an insulated container on ice. Do not mix seminal and ovarian fluid samples. For pooling restrictions see X B.4b and X C.

## VIII. TREATMENT OF SAMPLES

### A. AUTOPSY PROCEDURE

#### General Comments

The procedure outlined below is designed to facilitate the processing of large numbers of fish for the designated pathogens. Except for very small specimens, the same fish will serve as the source of tissues for the various bacteriological, virological, and myxosporean tests. The bacteriological examination must be performed first. To maximize detection sensitivity, fish must be autopsied within 48 h of sampling and all assay procedures should be initiated within this time.

#### External examination and sampling

Note all gross abnormalities such as body discoloration, body distension, exophthalmia, ulcers, blebs, inflammation, hemorrhagic areas, clubbed or necrotic gills, and eroded opercula, fins and caudal peduncles. Inoculate the appropriate media and prepare stained smears with material from these lesions.

#### Internal examination and sampling

Disinfect the surface of the fish and using aseptic techniques expose the kidney. Inoculate the appropriate media and prepare the appropriate smears with kidney material.

Inspect the viscera for abnormalities. Inoculate the appropriate media and prepare smears with material from any abnormal organs. Remove tissues for virological and myxosporean examinations.

### B. DISPOSAL OF SAMPLES

The receiving laboratory should handle and dispose of samples and other items likely to be infectious in a manner that precludes the dissemination of disease agents. All materials such as fish carcasses or tissues, transport containers and water, microbial cultures, and contaminated equipment should be autoclaved, incinerated, or otherwise sterilized before being discarded.

## IX. PROCEDURES FOR THE DETECTION OFCERTAIN BACTERIAL FISH PATHOGENS

### A. SCOPE

The microorganisms covered by these procedures are divided into three categories:

1. Those pathogens (listed in Schedule II) are considered to be limited in geographical distribution and the absence of which must be verified. These include the following certifiable disease agents:

   *Yersinia ruckeri* (Enteric redmouth disease)
   *Aeromonas salmonicida* (Furunculosis)

   Because it is neither cost effective nor biologically justifiable to continue looking for evidence of *Ceratomyxa shasta* from fish that do not show any clinical signs of disease (the parasite has, thus far, never been detected in Canada east of the Rocky Mountains) from fish that do not show any clinical signs of disease. Routine examination of stained smears of intestinal material taken from each fish collected for Fish Health Protection Regulations (FHPR) inspection is no longer required, except where the fish shows clinical signs of infection. Visual screening that reveals no clinical evidence of infection by *C. shasta* should be recorded by checking "not detected" in the Fish Health Certificate box. The "not tested" box is not checked off.

   If a client requires *C. shasta* screening for reasons over and above FHPR certification, this can still be undertaken under an agreement between the FHO and the client. A Fish Health Officer can choose whether or not to provide the diagnostic screening or prepare the slides for examination under an alternate arrangement. Clients should be informed *prior to each inspection* as to whether or not they require complete sample screening (i.e. examination of intestinal smears) for *C. shasta*, in order to avoid the need for them to kill more fish for such screening at a later date.

2. Those pathogens (listed in Schedule IV) that may be ubiquitous, and if detected during a disease outbreak or systematically in the absence of clinical signs in sampled fish, are notifiable. These include:

   Myxobacteria (e.g. *Flexibacter columnaris*)
   Bacterial Kidney Disease (*Renibacterium salmoninarum*) *
   Motile *Aeromonas sp.* (e.g. *Aeromonas hydrophila)*
   *Pseudomonas spp*.(e.g. *Pseudomonas fluorescens*)
   *Vibrio spp*. (e.g. *Vibrio anquillarum*)

   There is no longer a requirement to make, stain and examine kidney smears for Bacterial Kidney Disease (BKD) from fish collected during a Fish Health Protection Regulation (FHPR) inspection, except where fish show clinical signs of BKD. If there are suspected clinical signs of BKD, prepare and examine smears from the suspect kidney tissue, skin blebs

or muscle lesions, attempt growth of *R. salmoninarum* on a selective BKD growth medium, and take tissue samples for possible histological examination of disease.

If a client requires kidney smears from all fish to be screened for *R. salmoninarum*, for reasons over and above those of FHPR certification, this can be undertaken under an agreement between the FHO and the client. The FHO can choose whether or not to provide the diagnostic screening or to prepare slides for examination under an alternate arrangement. Clients should be informed *prior to each inspection* as to whether or not they require complete sample screening (i.e. examination of kidney smears) for BKD, in order to avoid the need for them to kill more fish for such screening at a later date.

3. Those bacterial agents not listed in Schedules II and IV, which the Fish Health Official detects and determines to be associated with significant losses and/or disease signs (e.g. *Edwardsiella tarda*).

**B. PROCEDURES**

The following procedures represent the minimum requirements in bacteriological testing; they must be applied to all samples taken from lots in which there is an unusually high prevalence of disease signs and/or mortalities. In samples from apparently healthy lots the procedures need only be applied to those fish averaging 4 cm or more in fork length (see VI A. 4a).

1. Aseptically obtain the following tissues/material and streak on the appropriate medium:

   a. Kidney tissue, preferably from sites that appear abnormal, and external/internal lesion material on Tryptic Soy Agar (TSA);

   b. Gill tissue and/or external lesion material on Shieh's medium (SH agar) or Cytophaga Agar (CA) only if gross pathology suggests a myxobacterial infection.

2. Prepare Gram-stained smears of kidney tissue and lesion material and examine a minimum of 25 fields (900-1000x magnification). The presence of small gram-positive diplobacilli, frequently present intracellularly, is presumptive evidence for *R. salmoninarum* (Sanders and Fryer 1980). (Presumptive evidence is strengthened by lack of growth on TSA.)

3. Incubate the TSA plates at $20^{o}$ C for five days: examine daily for growth. Incubate the SH or CA plates at 15-$20^{o}$ C for five days: examine daily for growth.

4. If gross pathology suggests a myxobacterial infection, prepare moist mounts of gill tissue and/or lesion material and examine for the presence of masses of long, thin rods. Select young representative colonies (yellow dry, rhizoid or yellow, moist, spreading) from the SH or CA plates and prepare Gram-stained smears. The presence of long, thin, Gram-negative rods, capable of gliding or creeping motility, constitutes a presumptive diagnosis for myxobacteria.

5. Select young representative colonies from TSA plates. Differentiate the microorganisms on the basis of the following characteristics:

    a. If the cells are Gram-negative, rod-shaped, oxidase-positive, non-motile, ferment glucose (O.F. test) and usually produce a brown diffusing pigment, the isolate is presumptively *A. salmonicida* (Griffin et al. 1952). Achromogenic strains of *A. salmonicida* may occur (Evelyn 1971).

    b. If the cells are Gram-negative, rod-shaped, oxidase, indole and $H_2S$-negative and produce an alkaline/acid (K/A) reaction in Triple Sugar Iron Agar (TSI), the isolate is presumptively *Y. ruckeri*.

    c. if the isolate differs from 5b.by being indole and $H_2S$-positive and produces both acid and gas in TSI, it is presumptively *E. tarda* (Amandi et al. 1982).

A flow chart is given for these procedures in which additional features separating motile aeromonads, *Pseudomonas spp*., and *Vibrio spp*. are indicated.

Confirmatory testing of presumptively identified certifiable agents must be performed. For a possible exception, see IX C 2g. The serological and biochemical tests described in IX C 2 are to be used for confirmatory testing.

Confirmatory tests for the notifiable agents may be performed at the discretion of the FHO. Identification should be based on whether or not the disease agent is associated with significant losses and/or gross pathology.

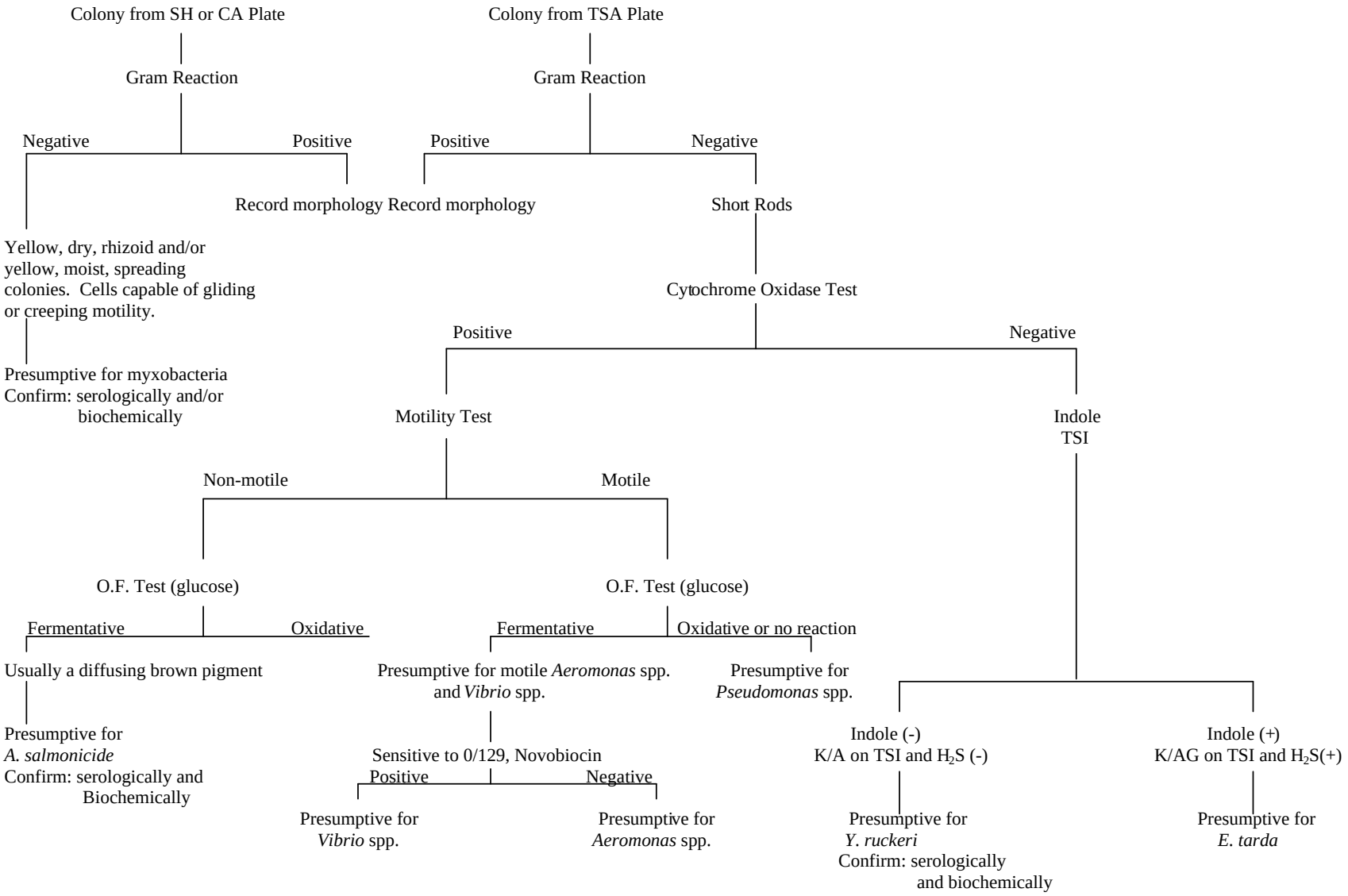


Figure 1. Differentation of bacteria isolates on TSA, SH or CA plates

## C. MATERIALS[3] and METHODS

### 1. Primary isolation media

a. Tryptic Soy Agar (Difco)

b. SH Agar (Shieh 1980)

| | |
|---|---|
| Agar | 1.0% |
| Peptone | 0.5% |
| Yeast extract | 0.05% |
| Magnesium sulphate | 0.03% |
| Sodium pyruvate | 0.01% |
| Monobasic potassium phosphate | 0.01% |
| Dibasic potassium phosphate | 0.005% |
| Sodium bicarbonate | 0.005% |
| Calcium chloride | 0.001% |
| Citric acid | 0.001% |
| Sodium acetate | 0.001% |
| Barium chloride | 0.001% |
| Ferrous sulphate | 0.0001% |
| pH | 7.0 |

**Note:** This is a modification of Shieh's medium. Neomycin (5 μg/mL) and polymyxin B (10 units/mL) can be added to SH agar to facilitate the isolation of myxobacteria by suppressing the growth of other bacteria (Fijan 1969).

c. Cytophage Agar (Anacker & Ordal 1959)

| | | | |
|---|---|---|---|
| Tryptone | 0.05% | Beef extract | 0.02% |
| Yeast extract | 0.05% | Agar | 0.9% |
| Sodium acetate | 0.02% | pH | 7.2 - 7.4 |

### 2. Test Media, Reagents and Methods

a. **Cytochrome oxidase test**: Using a platinum loop, transfer some bacterial growth from an actively growing plate culture to a paper strip impregnated with the appropriate chemicals. After thorough spreading, a positive test is indicated by the development of a bright blue colour within a minute. (MacFaddin 1980)

b. **Motility**: Examine log-phase cultures in wet preparations using Tryptic Soy Broth as the suspending medium. If the wet preparation method gives a doubtful result, check the results by stab-inoculating tubes of Motility Test Medium (Difco) or Glucose Motility Deep (GMD) medium (Walters and Plumb 1978).

[3] The products specified have proven satisfactory for the purposes indicated; this, however, does not imply that other products may not be equally satisfactory.

c. **Differentiation between oxidative and fermentative carbohydrate metabolism**: Perform an O.F. test (glucose) as described by MacFaddin (1980). Alternatively, inoculate tubes of GMD medium and interpret the results as described by Walters and Plumb (1978).

d. **Indole production**: Perform the test for indole production (MacFaddin 1980)

e. **Triple sugar iron agar**: The use of TSI agar and the interpretation of the results of this medium are described by MacFaddin (1980).

f. **Confirmatory slide agglutination test for *A. salmonicida* (Rabb et al. 1964) and *Y. ruckeri***: The agglutination test is performed by emulsifying a small amount of bacterial growth in saline (0.9% NaCl) on a clean glass slide. A loopful of antiserum is placed adjacent to the bacterial suspension and the two are mixed by gentle rocking and tilting of the slide. Prepare the appropriate positive and negative controls. A rapid macroscopic clumping of bacterial cells in the test mixture and the positive control (but not in the negative control) constitutes a positive agglutination test. Agglutination in the negative control invalidates the test. Many strains of *A. salmonicida* autoagglutinate. To prevent autoagglutination place the suspension in boiling water for 15 min prior to performing the slide agglutination test.

g. **Confirmatory tests for *R. salmoninarum***: If clinical signs or examinations of Gram-stained kidney smears suggest the presence of *R. salmoninarum*, retain a portion of the suspect kidney tissue and perform one of the following confirmatory tests. If such kidney tissue is unavailable, the presumptive evidence for *R. salmoninarum* noted earlier (IX.B.2) constitutes a confirmed diagnosis.

   **i) Immunodiffusion test** (Chen et al. 1974): Prepare immunodiffusion plates by adding 10 mL of medium consisting of Noble agar (1.0%), NaC1 (0.9%), and thimerosal (0.01%) to a 60 mm Petri plate. Punch a pattern of 6mm diameter wells, six of them peripheral to a centre well, so that all wells are 6mm apart. Place 0.1 mL specific antiserum in the centre well. Load the peripheral wells separately with 0.1 mL of saline (negative control), a heavy *R. salmoninarum* cell suspension in saline (positive control), and a 50% kidney homogenate in saline (test sample). Arrange the loading so test samples are adjacent to positive controls. Incubate the plates in a moist chamber at $15^{o}$ C for 48 h. A precipitin line of identity or partial identity between a test sample and a positive control constitutes a positive test.

   ii) **Direct fluorescent antibody test** (DFAT) (Bullock et al. 1980): Prepare a kidney smear on a clean glass slide, allow to air dry and fix for 5-8 min in acetone at $20^{o}$ C. Add 1-2 drops of the recommended optimal dilution of conjugated anti *R. salmoninarum* serum_containing a 1:150-1:200 dilution of rhodamine counterstain (Difco) to the slide and allow to react for 5-8 min at 20-$25^{o}$ C. Rinse the slide and wash for two min in phosphate buffered saline (pH 7.2) and air dry. Add a drop of mounting fluid (pH 9.0) to the test area, add a coverslip and examine a minimum of 25 fields under oil immersion using a microscope equipped with an ultraviolet light

source. A positive control should be prepared and stained in a similar manner. The presence of small fluorescing diplobacilli of typical size and shape constitutes a positive test.

iii) **Indirect fluorescent antibody test (IFAT)** (Bullock and Stuckey 1975): The indirect fluorescent antibody test can be used in place of the immunodiffusion test and DFAT to confirm the presence of *R. salmoninarum*. Consult the reference for the proper procedure.

h. **Biochemical confirmation of *A. salmonicida* and *Y. ruckeri***: Confirmatory testing of presumptively identified isolates of *A. salmonicida* and *Y. ruckeri* is performed using conventional media (Difco) as described by Edwards and Ewing (1972) or the API-20E miniaturized diagnostic system (bioMérieux SA 69280 Marcy l'Étoile, France) and comparing the results with those obtained for known (positive control) cultures of the disease agent.

i) Streak bacterial growth on TSA and incubate at 20° C for 24-48 h to obtain a pure culture.

ii) Prepare and inoculate separately the suspect bacterial culture and the known culture as recommended (Difco, API-20E). When confirming presumptively identified isolates of *Y. ruckeri*, a saline suspension having a final turbidity equivalent to that of a #1 McFarland turbidity standard is recommended as the inoculum.

iii) Incubate the inoculated biochemical test media at 20° C for 24-72 hours. Add the necessary reagents and read the test results as recommended (Difco, API-20E.)

iv) Compare the results obtained for the suspect isolate to those of the known bacterial culture(s). For interpretation of the biochemical profiles for *A. salmonicida* consult the papers by Paterson (1974) and Paterson et al. (1980); and for *Y. ruckeri* consult the paper by Stevenson and Daly (1982).

i. **Sensitivity to the vibriostatic agent 0/129 and the antibiotic novobiocin**: The test is performed on TSA plates by applying a 0/129 disk and a novobiocin (5µg) disk (Difco) to the medium that has been uniformly surface-seeded with the organism under test. After incubation at 20-22° C for 16-24 h, sensitive organisms show a clear zone around the disk. To prepare 0/129 disks, saturate Whatman antibiotic assay filter paper disks (6mm) with 0.1% 0/129 in acetone. Drain off excess solution and dry the disks at 37° C. The vibriostatic agent 0/129 (2, 4-diamino-6, 7-diisopropylpteridine) can be obtained from Oxoid Inc in Canada. A control disk impregnated only with acetone should be included to preclude the possible inhibitory reaction due to acetone.

j. **Agar block motility test for myxobacteria**:

i) Excise a 5mm square block of agar supporting a suspected myxobacterial colony. Place the block, colony side up, on a glass slide and cover gently with a cover slip.

ii) Examine the margin of the colony under high power for evidence of gliding or creeping motility.

k. **Confirmatory serological tests**: Confirmation of identity of bacteria by serological tests such as slide, tube, or micro-well agglutination reactions should be carried out using standard procedures. Sera used should be standardized, preferably absorbed and appropriate for the purpose. Positive and negative control organisms must be included.

## X. PROCEDURES FOR THE DETECTION OF VIRUSES

### A. SCOPE

1. Any filterable agent in the fish samples that replicates intracellularly in any of the specified cell lines is certifiable whether or not it can be identified with presently available antisera and whether pathogenicity for salmonids exists or is unknown. Methodology is dependent upon detection of cytopathic effects (CPE) in susceptible cell cultures.

2. Any abnormal proliferative lesions (tumours) encountered should be processed by histological methods, and the results of the histopathological evaluation reported.

### B. TISSUES TO BE ASSAYED

1. Sac fry: assay whole. When present, yolk sacs should first be removed and discarded.

2. Fish averaging 2-4 cm in fork length: remove and discard heads, but retain gills; cut off the tails just posterior to the vents. Mince the remainder of the carcasses and assay.

3. Fish averaging 4-10 cm in fork length: excise the gills, then eviscerate and assay the combined viscera and gills. After removal of the gills, evisceration is readily accomplished by first cutting off the head, then slitting open the body cavity from the cut end to the vent, and finally cutting and scraping to remove the viscera (including kidney).

4. a) Fish averaging 10 cm or more in fork length: assay mixtures of kidney, spleen, pyloric caeca-pancreas, and gills. The appropriate relative volumes of these tissues should be 3:1:1:1, respectively. One part each of anterior, central and posterior kidney must be represented in the sample.

   b) When the fish in this size category are brood fish and reproductive fluids are to be used, as many as possible of the reproductive fluid samples must be from female fish. For maximum sensitivity, assay reproductive fluid samples individually.

### C. POOLING

Tissues from a maximum of five fish may be pooled to form one sample. But when preparing pooled samples, apparently healthy fish (or their tissues) must not be pooled with dead and moribund fish (or their tissues).

### D. PREPARING INOCULA

Samples must be processed within 48 hours of collection (see VIII A.1). Prior to and during processing, samples must be kept refrigerated or on ice, but not frozen.

1. Solid tissues: weigh and then homogenize the tissues in a minimal volume of balanced salt solution (BSS) such as Earle's or Hanks' BSS at pH 7.6-7.8. Three methods are available:

    a. Homogenize using a stomacher.

    b. Use a sterile Ten Broeck, or homogenizer designed to allow cooling on ice during homonegization to process small fish or small amounts of tissue. Care should be taken to prevent possible aerosol dissemination of virus.

    c. Use a sterile pre-chilled mortar and pestle to grind the tissues with a small quantity of sterile sand (80-120 mesh silica) until a smooth paste is formed. Equipment used for homogenization of pooled samples within any given lot need not be sterilized between each use.

    After tissues have been triturated, dilute each sample extract to a final concentration of 2% tissue suspension in BSS. Centrifuge the extracts at 2500 x g for 15 min at $4^{o}$C and aseptically filter the clarified supernatant through a 0.45 μm pore diameter membrane filter. To avoid any appreciable loss of virus by absorption on the filter, collect the maximum possible volume of filtrate.

2. Fluid samples: dilute 1:2 with cold Eagle's minimum essential medium (MEM) at pH 7.2-7.6. Centrifuge at 2500 x g for 15 min at $4^{o}$ C. Decontaminate as in X D 1.

**E. ASSAY**

**Cell Cultures**

For virus assays to detect viruses listed under Schedule II, two of the four recommended continuous cell lines must be used: rainbow trout gonad (RTG-2), Chinook salmon embryo (CHSE-214), epithelioma papulosum cyprini (EPC) or fathead minnow (FHM). The EPC or FHM cell lines must be used in IHN virus enzootic areas. For other filterable replicating agents of concern capable of causing cytopathic effects in the cell lines of fish, cell lines may be used that are scientifically accepted and/or specified in the Manual of Diagnostic Tests for Aquatic Animals of the Office Internationale des Epizooties (OIE).

Using Infectious Salmon Anemia (ISA) as an example of a disease previously not detectable with the approved cell lines, the change allows the use of the SHK cell line which has been shown to support the detection of the virus. By being able to use this and other cell lines which are scientifically accepted and/or specified by the OIE, Canadian farms can be assisted with the export of live eggs and fish to countries that require screening for OIE listed diseases.

The amendment retains the requirement to use a minimum of two cell lines for all tests done under the FHPR, including the use of EPC or FHM cells in IHNV enzootic areas. Where needed for export purposes by a farm or for surveillance/monitoring to establish or maintain fish health zones, additional cell lines may be used.

Biannually or before each inspection season all stock cell cultures should be tested and found to be free of myoplasma. Each cell type must also be tested for susceptibility to viruses enzootic to the region (i.e., state, province, or watershed). The donor cell cultures used to prepare monolayers for virus detection must be no older than two weeks.

Additional information on fish cell culture and virology can be found in McAllister (1979), Pilcher and Fryer (1980), Wolf and Quimby (1969) and Wolf (1970).

**Either of the following procedures may be used**

a. Inoculation of preformed monolayers:

   i) Prepare duplicate monolayers of each of two cell lines for each sample to be tested. Plastic multidishes suitable for tissue culture (1.5 to 2.0 cm diameter wells) may be used sealed or unsealed (with the appropriate organic buffer incorporated in the medium).

   ii) Use 1.0 mL per well of Eagle's MEM at pH 7.6-7.8 containing Earle's salts, glutamine, and 10% fetal bovine serum (FBS). Alternate antibacterial mixtures for use in the medium are 100 IU penicillin/mL and 100 μg streptomycin/mL, or 50 μg gentamycin/mL. The use of a fungistat (e.g. 25 IU nystatin/mL) is also permitted.

   iii) Incubate the cell cultures at 15-20$^{o}$ C; the temperature depends upon when they will be required for the assay. At the time of inoculation the cell monolayers must be 70-90% confluent and not more than as 48 h old.

   iv) The growth medium must be removed and the monolayers washed with BSS, which is removed prior to inoculating 0.1 mL of the filter sterilized sample into each well.

   v) Incubate the inoculated cell cultures at 15$^{o}$ C for 60-90 min. Every 20 min gently rock cultures to uniformly spread the inocula. Add one mL of Eagle's MEM containing 2% FBS to each monolayer. Note: the new medium should be of the same composition as in E 2.a ii, except for decreased FBS and the final pH must be 7.6 to 7.8.

   vi) Incubate cultures at 15$^{o}$ C.

b. Simultaneously applied cells and test sample:

   i. Place in tissue culture wells 1.0 mL of medium (see X E 2. a.) containing sufficient cells to produce a 70-90% confluent monolayer on attachment. Duplicate cultures of two cell lines (see X E. 1.) are required per sample.

   ii. Immediately add 0.1 mL of the filter sterilized sample to each culture.

   iii. Incubate cultures at 15$^{o}$ C.

**Controls**

For each batch of donor cell cultures, duplicate negative controls must be run. Negative controls must consist of cultures inoculated according to the procedure used for the assays, except that sterile BSS must be used in place of the sample.

**Procedures to be followed during incubation**

a. Inspect the cultures shortly after inoculation and after 24 h. Thereafter examine cultures at least every day to determine whether CPE has been produced.

b. A sample is considered negative if there is no CPE in the cultures 14-21 days postinoculation.

c. If CPE occurs in one or more of the cultures inoculated with the samples, the presence of a filterable, replicating agent must be verified. Filter (0.45 μm pore diameter) the culture fluid from the test well(s) showing CPE, dilute the filtrate with BSS to $10^{-1}$ and $10^{-3}$, and inoculate 0.1 mL of each of the dilutions into fresh duplicate cultures of the same cell line. If CPE is again observed, proceed with the serum neutralization test.

## F. SERUM NEUTRALIZATION TEST

Presumptive identification of the agent producing CPE may be made on the basis of clinical evidence at sampling and on the type of CPE produced. Identification is accomplished by neutralization of the agent with specific antiserum. Failure to obtain any degree of neutralization using antisera prepared against known viruses will usually indicate the presence of a previously unrecognized virus or an atypical serotype of a known salmonid virus.

**Procedure**

a. Use a dilution of antiserum sufficient to neutralize an equal volume of a suspension containing $10^{2}$-$10^{3}$ $TCID_{50}$ per mL of the homologous virus.

b. Filter the fluid from a culture showing CPE through a 0.45 μm pore diameter membrane filter. Dilute the filtrate $10^{-2}$ and $10^{-6}$ with sterile BSS.

c. (1) Mix 0.3 mL of normal serum with 0.3 mL of each of the dilutions of sample.
(2) Mix 0.3 mL of normal serum with 0.3 mL of each of the dilutions of test sample.
(3) In the same manner, perform the serum neutralization test on the positive controls using homologous antiserum and normal serum.

d. Incubate the reaction mixtures at $15^{o}$ C for 30-60 min and then inoculate 0.2 mL of each mixture into duplicate cultures of the cell line in which the virus was isolated.

e. Incubate the cultures at $15^{o}$ C and observe for the production and inhibition of CPE. Inhibition of CPE by a particular antiserum, but not by normal serum, identifies the virus.

## G. OTHER CONFIRMATORY METHODS

The approved methodology for detection of viruses is based upon isolation, followed by serological identification. Methods of confirming the identification of the cell culture isolates are not limited to the suggested serum neutralization test (X F). Other acceptable immunoserological tests may be used, including fluorescent-antibody microscopy, immunoperoxidase techniques, enzyme-linked immunosorbent assays, microtitration and microneutralization, plaque neutralization, complement fixation and immunoelectron microscopy.

## XI. PROCEDURES FOR THE DETECTION OF CERTAIN PARASITES

### A. SCOPE

The absence of two myxosporean disease agents must be verified. These agents are *Myxobolus cerebralis* and *Ceratomyxa shasta*. Any other parasites detected which the Fish Health Official considers important should be reported.

### B. PROCEDURES FOR MYXOBOLUS CEREBRALIS

1. Fish for this procedure must be at least 120 days old, and preferably fresh. Frozen, but not formalin-preserved specimens may also be used. All glassware and equipment used in processing samples must be carefully cleaned to avoid carry-over of spores.

2. Decapitate fish and deflesh heads after heating in water at 45-50$^{o}$ C until the brain has coagulated. Remove the brain (and any attached spinal cord) intact. Discard to avoid possible contamination of skull material with spores of the related parasite *Myxobulus neurobius* that might be mistaken for *M. cerebralis*.

3. Either of the following procedures may be now used:

   a. Digestion Method[4]

      i) Pool up to approximately 100 g of the resulting skull material and macerate finely. Small initial fragment size will facilitate complete and rapid digestion.

      ii) Place in a beaker and add 25 mL of freshly prepared pepsin digest solution (1.0g of pepsin dissolved in 100 mL of 0.5% HC1) to each gram of macerated material.

      iii) Mix well, let settle for 2 min and examine a sample from the surface of the supernatant at 400-450X magnification for typical spores using phase contrast microscopy.

      iv) If spores are not detected, incubate the mixture at 35-40$^{o}$ C for 1-1.5 h with gentle agitation. A cloudy, grayish suspension, free of large particles, should be present at the end of the digest period.

      v) Place 50mL of the suspension into conical, screw cap centrifuge tubes designed to hold 50mL. Centrifuge at 1200 x g for 15 min at room temperature. Discard the supernatant and resuspend the pellet in 1.0mL of distilled water. The contents of up to five tubes can be combined into one sample. Examine microscopically for spores as before.

[4] The procedure outlined above is a modification of the pepsin-trypsin digest method of Markiw and Wolf (1974a and 1974b).

vi) If no spores are detected, bring the volume of each sample to approximately 6mL with distilled water. Layer the contents of each tube onto 3mL of 55% aqueous glucose solution contained in a 12mL conical centrifuge tube. Centrifuge at 1200 x g for 30 min at room temperature.

vii) Withdraw pelleted material with a Pasteur pipette and examine at least 25 microscope fields per sample as given in XI B 3a. (iii). Observation of spores at any stage in the procedure constitutes a positive result.

b. Plankton centrifuge method (O'Grodnick 1975 and Prasher et al. 1971)

i) Pool up to 100 g of skull material and macerate in a blender for five min with distilled water. Up to 200 mL of water per 100 g of skull material can be used.

ii) Remove the macerated material and vacuum filter through a fine (0.5-1.0 mm) wire mesh. If the filter clogs, rinse with distilled water to clear it allowing the rinse water to mix with the filtrate. Coarse material such as large bone chips are removed by this procedure and can be discarded.

iii) Place the filtrate in a separatory funnel located to discharge into a plankton centrifuge. Run the centrifuge at high speed while adding the filtrate in slowly.

iv) Centrifuge until all water has been removed. Scrape the residue from the wall of the centrifuge and place in a small bottle. Add five volumes of distilled water. Do not dilute beyond a total of 30 mL. Cap and shake the bottle until the material is uniformly suspended.

v) Place a drop of the suspension on a slide or hemocytometer if quantification is required.

vi) Examine at least 25 fields of the slide at 450X magnification for the presence of *M. cerebralis* spores using phase contrast microscopy.

4. Confirmatory identification of *M. cerebralis* spores must be based on the morphological characters given by Lom and Hoffman (1971)

## C. PROCEDURE FOR *CERATOMYXA SHASTA*

1. Fish for the following procedures must be at least 120 days old; either fresh (preferably) or frozen specimens may be used.

2. The preferred organs for examination for *C. shasta* spores are the intestine and gall bladder. Peritoneal fluid may also contain spores. Microscope slides of tissue, fluid and purulent material can be prepared and examined as required by either of two methods:

a. **Wet mounts**: Prepare wet mounts by gently mixing sufficient material in one or two drops of saline (0.9% NaCl) on a standard microscope slide to give a reasonably dilute suspension, cover with a cover slip and examine at 400-450x magnification, using phase contrast microscopy.

b. **Dried smears**: Smear material to be examined on a standard microscope slide, allow to air dry, stain 30-60 sec with Loeffler's methylene blue (dissolve 0.3 g of methylene blue chloride in 30 mL of 95% ethanol and add 100 mL of 0.01% aqueous KOH), rinse with water and air dry. Add a drop of immersion oil or water to each smear, cover with a cover slip and examine at 400-450x magnification for the presence of spores. The polar capsules and extended polar filaments stain an intense blue and the sporoplasm stains a pale blue.

3. For fish less than 7.5 cm in fork length, material for examination can be expressed from the intestines. In addition, if nodular lesions are present in any tissue, or if ascetic fluid is evident, prepare smears or wet mounts of this tissue or fluid.

4. For fish 7.5 cm or more in fork length, open the body cavity and collect material lightly scraped from the interior wall of the upper intestine or gall bladder. If nodular lesions are observed on or in any tissue, especially the pyloric caeca, or if any abnormal accumulations of fluid are found, they must be examined for spores of *C. shasta*.

5. For each smear or wet mount at least 25 microscope fields must be examined for spores of *C. shasta*.

6. The identification of *C. shasta* spores must be based on the diagnostic characters given by Johnson et al. (1979). Pre-spore stages of *C. shasta* may be found without accompanying spores; by themselves they are not diagnostic for the organism and their presence indicates that further samples and search for the characteristic spores should be made (Noble 1950; Yamamoto and Sanders 1979).

Additional information on identification of fish disease agents can be found in McDaniel (1979).

## XII. EGG DISINFECTION PROCEDURES

Salmonid eggs are safely disinfected as green eggs following fertilization and water hardening, or as early eyed eggs. The following is a suggested procedure for egg disinfection, utilizing iodophors. Iodophors for disinfection are usually povidone or polyalcoholic complexes of iodine in which the solubilized iodine confers its broad spectrum germicidal activity, but is not as corrosive or irritating as in its elemental form. A number of topical disinfectants[5] of this type are available commercially in North America; among these are Ovadine®, Bridine®, Betadine®, Actomar K30®, Wescodyne® and Argentyne®. Most contain a 1-2% active iodine concentration.

### A. PREPARATION OF THE DISINFECTANT

1. Dilute the stock iodine-based disinfectant to give a solution containing 100 parts per million (ppm) of active iodine. The disinfectant must be prepared in water with a low organic content to minimize loss of the free iodine. Use a plastic, glass, stainless steel or fibreglass tank for preparing the holding of the solution.

2. Check the pH of the diluted disinfectant and, if necessary, adjust to 6.5-7.5 using 8% aqueous sodium bicarbonate (baking soda).

### B. DISINFECTION PROCEDURE

1. Use a fresh solution of diluted disinfectant.

2. To avoid temperature shock, adjust the disinfectant solution to the same temperature as the subsequent egg incubation temperature.

3. In the case of freshly fertilized eggs, allow eggs to water harden one hour before disinfection.

4. Immerse water hardened green eggs or early eyed eggs in the disinfectant for ten min.

5. Treat approximately 2000 eggs per litre before discarding the disinfectant.

6. Rinse eggs thoroughly in uncontaminated water after disinfection.

7. Arrange the egg handling program to ensure that disinfected eggs do not have subsequent contact with contaminated equipment, water or personnel.

Diluted iodophors can also be used to disinfect work surfaces, utensils, nets and other equipment used during the egg taking process, but rinse thoroughly in clean, uncontaminated water following the disinfection.

[5] The products specified have proven satisfactory for the purposes indicated; this, however, does not imply that other products may not be equally satisfactory.

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## APPENDIX 1

## NATIONAL REGISTRY OF AQUATIC ANIMAL HEALTH

The National Registry of Aquatic Animal health is a data centre for the documentation and dissemination of information pertaining to fish diseases in Canada. Fish Health Laboratory Reports of all disease examinations in Canada, not only those specifically for certification purposes but also those from routine periodic sampling programs should be submitted routinely to the Registry by Fish Health Officials.

The National Registry of Aquatic Animal Health will:

1. maintain close watch on geographic distribution and prevalence of fish diseases in Canada and identify emerging disease problems;

2. serve as a coordinating center in the event of national fish health emergencies;

3. provide periodic reports, analyses, and assessments on the state of fish health in Canada;

4. provide health histories of sources of live fish and eggs;

5. maintain and provide lists of certified Canadian and foreign fish production facilities;

6. maintain and provide lists of Local Fish Health Officers and Fish Health Officials approved by the Minister; and

7. maintain and provide lists of Canadian government approved, certifying officials in foreign countries involved in export of fish and eggs to Canada.

The address for the Registry is:

The National Registry of Aquatic Animal Health
Fisheries and Oceans Canada
Ottawa ON Canada
K1A 0E6
Tel. 613-949-7522 Fax: 613-993-7665 E-mail: nrfd@dfo-mpo.gc.ca

## APPENDIX 2

## REGIONAL ADMINISTRATIVE AUTHORITIES

The Fish Health Protection Regulations are administered and applied by various provincial and regional authorities. In some areas provincial authorities are involved while other areas are a federal responsibility. The addresses follow:

## PROVINCIAL FISHERIES AUTHORITIES

| | | |
|---|---|---|
| ALBERTA | Alberta Fish and Wildlife<br>6909 – 116 Street<br>Edmonton, AB T6H 4P2 | Tel.: 780-427-8288 |
| BRITISH COLUMBIA | BC Ministry of Water, Land and Air Protection<br>PO Box 9363, Stn PROV GOVT<br>Victoria BC V8W 9M2 | Tel.: 250-387-9500 |
| | BC Ministry of Agriculture, Food and Fisheries<br>Access Centre, 2500 Cliffe Ave<br>Courtenay, BC V9N 5M6 | Tel: 250-897-7500 |
| MANITOBA | Manitoba Dept of Natural Resources<br>Box 48<br>Saulteaux Crescent<br>Winnipeg MN R3J 3W3 | Tel.: 204-945-7789 |
| ONTARIO | Ontario Ministry of Natural Resources<br>Fish and Wildlife Branch<br>300 Water Street<br>Peterborough, ON K9J 8M5 | Tel: 705-755-1928 |
| QUÉBEC | Direction du développement de la faune<br>Société de la faune et des parcs du Québec<br>Édifice Marie-Guyart, 11 étage, boîte 92<br>675, boul. René-Levesque Est<br>Québec QC G1R 5V7 | Tél : 418-521-3875<br>poste 4496 |
| SASKATCHEWAN | Saskatchewan Environment<br>3211 Albert Street<br>Regina, SK S4X 5W6 | Tel.: 306-787-2467 |

## REGIONAL FEDERAL FISHERIES AUTHORITIES

| | | |
|---|---|---|
| ONTARIO, MANITOBA, SASKACHEWAN, ALBERTA, NUNAVUT TERRITORY | Fisheries and Oceans Canada<br>Freshwater Institute<br>501 University Avenue<br>Winnipeg MN R3T 2N6 | Tel: 204-983-5125 |
| NEWFOUNDLAND | Fisheries and Oceans Canada<br>Newfoundland Regional Office<br>P.O. Box 5667<br>St. John's NL A1C 5X1 | Tel: 709-772-2891 |
| NORTHWEST TERRITORIES | Fisheries and Oceans Canada<br>42043 Mackenzie Hwy<br>Hay River, NWT X0E 0R9 | Tel: 867-874-5575 |
| NEW BRUNSWICK<br>NOVA SCOTIA<br>PRINCE EDWARD ISLAND | Gulf Regional Office<br>Fisheries & Oceans Canada<br>P.O. Box 5030<br>Moncton, NB E1C 9B6 | Tel: 506-851-6081 |
| YUKON & MARINE AREAS OF BRITISH COLUMBIA | Fisheries and Oceans Canada<br>Pacific Biological Station<br>3190 Hammond Bay Road<br>Nanaimo BC V9T 6N7 | Tel: 604-756-7069 |

## APPENDIX 3

## QUALIFICATIONS OF FISH HEALTH OFFICIALS

A Fish Health Official carries the burden of a great deal of responsibility and therefore must be knowledgeable in the field of fish health and thoroughly familiar with the methodology for diagnosing fish diseases and their causative infectious agents. The following criteria represent the minimum requirements for acceptance by the Canadian Government as a Fish Health Official.

### A. EDUCATION AND EXPERIENCE

1. Bachelor's degree or equivalent in any field of fisheries or biological science and two years' experience in the diagnosis of fish diseases, including experience in bacteriological, virological, and parasitological techniques.

2. Veterinary, master's, or doctoral degree or equivalent with evidence of training in bacteriology, virology, and parasitology and one year's experience in the diagnosis of fish diseases.

### B. LABORATORY FACILITIES

Laboratory facilities adequate for microbiological and parasitological evaluation of samples must be available to the proposed Fish Health Official.

### C. APPLICATION PROCEDURE

To be considered for approval as a Fish Health Official, a candidate must submit a curriculum vitae, three specimen signatures, and a description of available laboratory facilities and equipment to the National Registry of Aquatic Animal Health, and complete a knowledge based questionnaire.

## APPENDIX 4

## FISH HEALTH CERTIFICATE

The fish health certificate has been revised. Please note the following changes that have been made to the form:

1) The certificate must be numbered. This will assist in tracking that the most current certificate is used. It may be required for exports to countries outside of Canada.
2) Because the requirements for testing differ, the new form distinguishes between facilities certified for egg shipments only and those certified for eggs and fish.
3) The listing of "other pathogens" has been dropped. If pathogens of note are detected in the course of employing methods of the Manual of Compliance and its amendments (e.g. cell line amendment), they should be noted in the fish health laboratory form and entered in the "Notes" section of the Certificate.
4) A wording change has taken place to indicate that the four most recent inspections are to be noted starting with the inspection on which the certificate is based.
5) The size has been changed to the 8.5x11 format to ease photocopying of the form.

Fisheries and Oceans Pêches et Océans

Certificate N°. _____

# FISH HEALTH CERTIFICATE

**Eggs Only** ☐ **Fish and Eggs** ☐

Name of facility/source: _____
Address: _____
Telephone N°. _____ Fax N°. _____ Email _____

I, _____, as a Fish Health Official under the Canadian Fish Health Protection Regulations C.R.C., c.812, certify that the source indicated above was inspected by the methods approved by the Minister of Fisheries and Oceans Canada and that the following pathogen status was determined as required by those Regulations.

| **Pathogen** | **Detected** | **Not Detected** | **Not Tested** |
|---|---|---|---|
| Viral Hemorrhagic Septicemia Virus | ☐ | ☐ | ☐ |
| Infectious Hematopoietic Necrosis Virus | ☐ | ☐ | ☐ |
| Infectious Pancreatic Necrosis Virus | ☐ | ☐ | ☐ |
| Other filterable replicating agent | ☐ | ☐ | ☐ |
| *Aeromonas salmonicida* | ☐ | ☐ | ☐ |
| *Yersinia ruckeri* | ☐ | ☐ | ☐ |
| *Myxobolus cerebralis* | ☐ | ☐ | ☐ |
| *Ceratomyxa shasta* | ☐ | ☐ | ☐ |

Notes: _____

**Date of the last four inspections: use D/M/Y notation**

_____ _____ _____ _____

| Date of Issue | Signature of Fish Health Official | Affiliation & Postal Address | Telephone N°./Fax N° |
|---|---|---|---|
| _____ | _____ | _____ | _____ |

This certificate expires on the date the pathogen status changes or _____ (D/M/Y), whichever is the earlier.

## EXPORTER'S DECLARATION

I, _____ , ☐ owner ☐ manager of the above noted facility which was last inspected on _____(D/M/Y) declare that, to my knowledge, no disease agent(s) listed in Schedule II of the Fish Health Protection Regulations (FHPR) that would alter the above described pathogen status have been detected, in this facility, according to the procedures outlined in the FHPR Manual of Compliance since the last FHPR inspection; that no introduction of fish or fish eggs from any source that would alter the above pathogen status has been made into the facility; that the shipment described below will be derived solely from this facility; and that eggs in the shipment will be surface disinfected prior to leaving the source.

I, _____ , consignor of eggs taken from wild spawners declare that these eggs will be surface disinfected and that they derive solely from the above inspected source.

**This shipment consists of:**

_____ kg ☐ Live ☐ Eggs Species: _____
_____ Number ☐ Dead ☐ Fish Species: _____

_____ _____ _____

| Date | Signature and Address of Owner, Manager or Consignor | Telephone No. |
|---|---|---|

## IMPORTING INFORMATION

Departing city and country _____ Carrier _____
Bill of lading No. _____ Date _____
Anticipated port of arrival in Canada (City and Province): _____ Date _____(D/M/Y)

_____ _____ _____

| Date (D/M/Y) | Signature and Address of Importer | Telephone No. |
|---|---|---|

## APPENDIX 5

## FISH HEALTH LABORATORY REPORT

This report form has been revised entirely. An example is attached to assist you with completing the new form. The form allows detailed review of the diagnostic methods and results. The additional information recorded can be used by the Local Fish Health Officer in reviewing an application for an import permit. Even though a range of tests is listed, the methods of the Manual of Compliance and its amendments are the standard upon which Canadian import permits are based. The change allows flexibility to use this Laboratory Report form for fish health certification purposes other than FHPR, e.g. for OIE-based trade requirements.

Government of Canada Gouvernement du Canada
Fisheries and Oceans Pêches et Océans

# Fish Health Protection Regulations Laboratory Report

Sheet of

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**Name of Facility:**
**Address:**

**Effluent Drainage:**

**Water source:**

**Watershed name:**

**Telephone**

**Effluent or influent treatment: (specify)**

**Prior Inspections Dates:**
**(previous 24 months)**
**(dd/mm/yy)**

**Current Inspection Date**
**(dd/mm/yy)**

| Species | Lot ID | Age | Number in lot | Obtained as Eggs (E) or Fish (F) from (Source) | Date of Introduction (dd/mm/yyyy) | *Pathogens-Methods and Results | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | OMV | IPNV | IHNV | VHSV | ISAV | Yr | As | Rs | Mc | Cs |
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**Samples Collected By:**
**Affiliation:**

**Diagnostic Laboratory:**
**Supervising Fish Health Official:**

**Laboratory Address:**

**Signature of Fish Health Official**_____
**Date:**_____

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## FOOTNOTES:
## SPECIES ABBREVIATIONS:

| | |
|---|---|
| ARC | Arctic char (Salvelinus alpinus) |
| ARG | Arctic grayling (Thymallus arcticus) |
| ATS | Atlantic salmon (Salmo salar) |
| BKT | Brook trout (Salvelinus fontinalis) |
| BNT | Brown trout (Salmo trutta) |
| CHS | Chum salmon (Oncorhynchus keta) |
| COS | Coho salmon (Oncorhynchus kisutch) |
| CKS | Chinook salmon (Oncorhynchus tshawytscha) |
| CUT | Cutthroat trout (Oncorhynchus clarki) |
| DOV | Dolly Varden trout (Salvelinus malma) |
| HYS | Hybrid salmon or trout (specify cross) |
| KOE | Kokanee (Oncorhynchus nerka) |
| LAT | Lake trout (Salvelinus namaycush) |
| OSA | Other salmonid species (Innconnu, Plecoglossus, Hucho, Brachymystax, etc specify _____) |
| PKS | Pink salmon (Oncorhynchus gorbuscha) |
| RBT | Rainbow trout (Oncorhynchus mykiss) |
| SOS | Sockeye salmon (Oncorhynchus nerka) |
| STT | Steelhead trout (Oncorhynchus mykiss) |
| WHF | Whitefish (Coregonus, Prosopium, etc) specify Genus and species_____) |
| */T | species abbr./Transgenic |

_____

Age is counted from hatch. In lots of fish less than one year of age, the age is listed in Arabic numerals followed by mo. for month; for fish older than one year, the age is expressed in Arabic numerals followed by yr.

_____

Findings are reported in columns from top to bottom for each lot as follows: Box 1: number of fish examined; Box 2: methods used; Box 3: results (negative or prevalence of infection plus confirmatory test used).

_____

## PATHOGEN ABBREVIATIONS:

| | |
|---|---|
| IPNV | Infectious Pancreatic Necrosis virus |
| IHNV | Infectious Hematopoietic Necrosis virus |
| VHSV | Viral Hemorrhagic Septicemia virus |
| OMV | Oncorhynchus masou virus |
| ISAV | Infectious Salmon Anemia virus |
| As | Aeromonas salmonicida |
| Yr | Yersinia ruckeri |
| Rs | Renibacterium salmoninarum |
| Mc | Myxobolus cerebralis |
| Cs | Ceratomyxa shasta |

## A. Prevalence of infection

c = carriers
i = clinical infection
e = epizootic

## DIAGNOSTIC METHODS:

**VIRAL PATHOGENS:** Methods encoded as follows:
First letter = sampling method

A = whole fry homogenates
B = whole visceral homogenates
C = kidney/spleen
D = reproductive fluids
E = kidney/spleen/pyloric caeca/gill lamellae
F = kidney/splee/encephalon
G = other

Numbers = continuous cell lines used

1 = RTG-2 (rainbow trout gonad)
2 = CHSE-214 (chinook salmon embryo)
3 = FHM (fathead minnow)
4 = EPC (epithelioma papillosum cyprini)
5 = BF2 (bluegill fin)
6 = SHK-1 (salmon head kidney)
7 = other cell lines

Last letter = Pooling of samples

A = individual fish
B = five fish pools
C = Other_____

**BACTERIAL PATHOGENS:** Encoded as follows:

Letter= Health of fish sampled
A= live, random
B= moribund
C= Mortalities

Number = Material sampled
1 = kidney
2 = lesion
3 = gill
4 = Other_____

Last letter = technique used for:

Primary Isolation

A = Standard culture medium TSA
B = Cytophaga agar
C = Shieh's medium
D = Other_____

Presumptive Diagnosis
E = Visual inspection only (Rs)
F= Gram stain, kidney smears (Rs)
G = Standard biochemical/physical testing
H= Other_____

**PROTOZOAN PATHOGENS**: Encoded as follows:

A = Digestion method
B = Plankton centrifuge method
C = Examination of stained smear
D = Visual inspection only (Cs)

## B. CONFIRMATORY TESTING FOR VIRAL, BACTERIAL, & PARASITIC PATHOGENS

H = Serum neutralization
I = Fluorescent antibody test
J = Agglutination (Slide, tube, micro-well)
K = ELISA
L = Biochemical profile
M = PCR
N = Other_____

_____

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