



Sample collection and submission for fish virus isolation by culture

Pilchard orthomyxovirus (POMV), Tasmanian salmon aquareovirus (TSRV) and Tasmanian aquabirnavirus (TABV) are viral pathogens which can cause serious disease in farmed salmonid fish. Routine testing for the presence of POMV, TABV and TSRV is by polymerase chain reaction (PCR), a molecular diagnostic test.

Where there is a suspicion of viral disease, but known viruses have not been detected by PCR, virus isolation by tissue culture can be used. This approach is particularly important when investigating new or unusual disease events.

Purpose

Collection and submission of samples for fish virus isolation requires planning and the use of specialised sampling techniques. Procedures and requirements for sampling are described in this Lab Fact.

Planning

Salmonid viruses are grown on fish cell lines which are made ready from laboratory stocks held for this purpose. It takes up to 7 days to propagate and prepare the cell lines ready for inoculation.

Before sending in samples for virus culture, first contact the Animal Health Laboratory to make a booking. We can advise on the type and number of cell lines which should be used for the investigation and the most appropriate means of sample collection and submission.

Even if cell lines are not prepared and ready for inoculation, samples should still be collected and submitted to the Animal Health Laboratory

where they will be held until the cell lines are ready for inoculation.

Selecting animals for testing

Only moribund, live fish should be selected. Samples from dead fish are unsuitable for virus isolation.

Number of animals to be tested

At least 5 animals should be selected either for field sampling or submitting as whole fish on ice.

Approach to sample collection

A key factor in successful virus isolation is sample integrity. Contamination during sample collection will prevent virus growth on fish cell lines.

Strict aseptic procedures are essential for collecting samples for virology. With the right conditions and procedures, it is possible to collect good samples at a farm field station or laboratory. It is highly recommended that training is obtained from the Animal Health Laboratory before attempting sampling in the field.

If the affected fish are small (50 grams or less), or field sampling cannot be done with confidence, chilled fish, packed on ice, can instead be sent to the Animal Health Laboratory.

In most cases, whole fish on ice are the preferred type of sample for submission.

What samples to collect

Most of the known viruses of salmonids in Tasmania can be isolated successfully from pooled liver, kidney and spleen samples. If infection of other organs (e.g., gills, brain, gut) is suspected, these tissues should be submitted in separate containers for each fish. The Animal Health Laboratory can advise on which organs should be sampled.

Materials required

Field sampling

- Paired sets of sterile dissection instruments.
- Isopropyl alcohol impregnated wipes for cleaning and disinfecting instruments.
- Sample collection tubes of viral transport medium – supplied by the Animal Health Laboratory on request.
- Insulated box with enough ice bricks to maintain samples at 2–4°C during transport to the Animal Health Laboratory.

Sending whole fish

- Crushed ice to maintain fish at 2–4°C during transport to the Animal Health Laboratory.
- Insulated box for packing fish.

Field Sampling Method

Dissection of fish for virology sampling should not be attempted on board a boat unless a dedicated inside sampling area is available. The preferred place for dissection of fish should be at a land-based field station or laboratory.

- a) Use one set of sterile instruments to expose the organs and the second set to collect liver, kidney and spleen samples.
- b) Use isopropyl alcohol wipes frequently during dissection and sampling to disinfect the two sets of instruments.
- c) For each organ, excise a 5-10 mm³ piece of tissue (about the size of a pea).
- d) Place each of the three tissues into a single tube of sterile viral transport medium.
- e) If sampling other tissues such as gill or gut, use a separate tube for each tissue.
- f) Clean and disinfect the instruments with isopropyl alcohol wipes between each fish sampled.

- g) **Do not pool samples** from different fish; use one tube for each fish sampled.
- h) Each sample bottle should be clearly labelled with the date of collection and fish identification (number and pen).

Packing Samples

Field tissue samples

- a) Keep samples chilled, on ice, after sampling and at all times during transport.
- b) Pack securely in an insulated box with pre-frozen ice bricks.

Whole fish

- c) Keep fish chilled, on ice, after collection and at all times during transport.
- d) Pack fish in plenty of crushed ice in an insulated box.

Transporting samples

Notify Specimen Reception when sending the samples. These must arrive at the Animal Health Laboratory within 24 hours of collection. Do not rely on Australia Post services for time critical samples.

Submitting samples

Samples should be submitted using a Fish Health Unit submission form. If forms are not available, an electronic form can be found at www.nre.tas.gov.au/ahlabsforms. Information about the purpose of the investigation should be provided along with the type and number of cell lines that are to be used for virus isolation.

For more information visit the AHL website

www.nre.tas.gov.au/AHLabs

Submit samples to:

Animal Health Laboratory

NRE Tas

165 Westbury Road

Prospect TAS 7250

Phone: 03 6777 2111

Email: specimenreception@nre.tas.gov.au

