

## 2.2.8.2 Infectious Salmon Anemia Appendix 2

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### Indirect Fluorescent Antibody Test for Infectious Salmon Anemia Virus (ISAV)

#### A. Principle/Purpose

To determine the presumptive presence/absence of ISAV in samples of fish tissue, blood, mucus, and/or body fluids.

#### B. Specimen

Most common samples tested consist of kidney tissue and blood. The former involves lethal sampling while the latter can be performed non-lethally. In both cases, a blot is prepared on a slide that is in turn used for the staining. Please refer below for procedures used in the preparation of blots.

#### C. Materials

Slides  
Forceps  
Scalpel  
70% isopropanol  
Bench paper  
PAP pen <sup>a</sup>  
5% solution of skim milk in sterile PBS  
 $\alpha$  ISAV monoclonal antibody (primary)<sup>b</sup>  
TRITC-labeled goat  $\alpha$ -mouse antibody (secondary)<sup>c</sup>  
Phosphate buffered saline (PBS) with 0.1% Tween; pH 7.2

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<sup>a</sup> Purchased from Ted Pella Inc., Redding CA

<sup>b</sup> Purchased from Dr. Knut Falk, National Veterinary Institute, Oslo, Norway

<sup>c</sup> Purchased from Sigma Chemicals, St. Louis, MO

## D. Procedure

### 1. Sample Preparation

- a. **Kidney Blot**  
Using sterile forceps and scalpel, remove a 1cm<sup>3</sup> piece of tissue from the mid-kidney. Firmly touch one surface of the kidney chunk two times onto a clean slide. Each blot should cover approximately 1/2 of the slide surface. The first blot will contain more blood than the second, however, avoid leaving a blot which is too thick or too thin.
- b. **Blood Sample**  
Place two 25 µL drops of blood onto a clean slide. Spread each blood sample, being careful to keep some separation between each drop.
- c. Allow sample to dry for 30 to 60 minutes and fix by immersing in acetone for 10 minutes. Store slides at -20°C until stained.

### 2. Staining

- a. If slides are removed from -20°C, allow to reach room temperature before staining.
- b. Trace each blot with the PAP pen. Allow to dry for a few minutes. Place 2 to 3 layers of paper towels on a plastic tupperware lid and moisten thoroughly with de-ionized water. Lay slides on the moist paper towels, making sure that they are level.
- c. Prepare a solution of 5% skim milk using sterile PBS (pH 7.2). The solution can be used for up to a week if stored at 2 to 8°C. Place 100 µL of this block solution per blot and spread to cover the whole blot using the edge of the pipet tip. Avoid touching the blot; change pipet tips if necessary.
- d. Cover slides with bottom of tupperware container and allow incubate for 30 minutes. Do not allow slides to dry during the staining procedure.
- e. Prepare a primary antibody staining solution of α ISAV monoclonal antibody by making a 1/100 dilution in sterile PBS (pH 7.2). Use laminar flow hood and aseptic technique while preparing the solution. The solution can be kept for several weeks at 2 to 8°C.
- f. Tap off skim milk solution by tilting each slide. Lay slides back on the moist paper towel and place 50 µL of primary antibody (α ISAV mAb) on each blot. The antibody solution should cover the blot without any need for spreading; check by viewing the slides at an angle to light. Cover with tupperware bottom and allow to incubate for 60 minutes.
- g. Tap slides on paper towels as before, dip in a solution of PBS with 0.1% Tween and soak in a second container of the same solution for six minutes. Either metal slide racks or plastic specimen cups may be used during the washes.
- h. The remainder of the staining procedure must be performed away from direct light.

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- i. Prepare a secondary antibody staining solution of TRITC labeled goat  $\alpha$  mouse antibody by making a 1/100 dilution in sterile PBS; prepare aseptically as before. The antibody solution can be kept for several weeks at 2 to 8°C.
- j. Place slides back on the moist paper towels. Dispense 50 mL of secondary antibody onto each blot and check to make sure it is spread evenly over the blot. Cover slides with tupperware bottom and incubate for 60 minutes.
- k. Repeat step g.
- l. Place slides face up on dry paper towels and allow to dry at room temperature. If slides are not going to be viewed immediately, place in a slide box and keep at 2 to 8°C until analysis.

### 3. Resulting Stained Slides

- a. View slides under fluorescence with a wide green filter after mounting a coverslip with 1 to 2 drops of immersion oil.
- b. The interpretive scoring method for IFAT/ISAV is as follows:
  - 1+ : sparse distribution of fluorescent grains or specks throughout
  - 2+ : more obvious distribution of grains, ( $>10 \text{ field}^{-1}$ ) e.g. around cell membranes
  - 3+ : obvious distribution of grains, e.g. broken lines around cell membranes
  - 4+ : broad fluorescence; 3 to 4 fully involved cells per field of view
- c. An interpretive scoring of 1+ or 2+ is considered a suspect rating result for ISAV by IFAT.
- d. An interpretive scoring of 3+ or 4+ is considered a positive rating result for ISAV by IFAT.