

## 4.4 Sample Processing Procedures

Tissue processing for viral culture is described below. Ingredients and preparation procedures for buffers and other solutions/media are listed in Section 2, 4.9 Reagents and Media (Amos 1985; OIE 2000; Rovozzo and Burke 1973; True 2000; Wolf 1988).

### A. General Considerations

1. As during sampling and transport, care is taken to protect tissues and fluids from exposure to UV light, freezing, or high temperatures lethal to the viruses of interest.
2. During sample processing, dilution levels are selected from the acceptable range to maintain maximal virus titers and minimize cell toxicity.
3. Following inoculation of test media, remaining tissue/fluid products are kept at 4°C until all assays are completed. Subsequent to the completion of the assays, all material is decontaminated and discarded.

### B. Processing of Kidney and Spleen Samples

1. If transport medium (Section 2.3.C.2 “Hanks Balanced Salt Solution (HBSS)”) is used, it is poured off and disinfected before discarding. Tissue samples are weighed to the nearest 0.1 g and sterile sample dilution medium (Section 2, 4.9.A “Sample Dilution Medium - Hanks Balanced Salt Solution (HBSS)”) is added to make a dilution of 1:10 to 1:100 (w/v). Unless there is a high potential for cell toxicity from the sample, the 1:10 dilution will be used.
2. Tissues are homogenized and a measured amount of homogenate is pipetted into a sterile tube for centrifugation.
3. Tubes are centrifuged at 4°C for 15 minutes at 2000-3000 times gravity relative centrifugal force (X g).
4. An aliquot of supernatant (for virologic evaluation) is transferred to a tube containing an equal amount of antibiotic incubation medium (anti-inc) (Section 2, 4.9.B “Antibiotic Incubation Medium (Anti-Inc) Made with HBSS for Sample Disinfection” or Section 2, 4.9.C “Antibiotic Incubation Medium (Anti-Inc) Made with Minimum Essential Medium (MEM-0) for Sample Disinfection”). Sample dilution is now 1:20 volume/volume.

Tubes are vortexed and incubated for two hours at 15°C or 12 to 24 hours at 4°C. Samples are then re-centrifuged at 2000-3000 X g and the supernatant is inoculated onto tissue culture plates as described in the inoculation procedures (Section 2, 4.5.A “Plate Inoculation Procedures for Primary Culture”).

### C. Processing of Coelomic (Ovarian) Fluid Samples

1. An equal volume from each ovarian fluid sample is pipetted into a sterile tube for centrifugation.
2. Centrifuge the tubes at 2000-3000 X g for 15 minutes at 4°C.
3. Undiluted ovarian fluid may be used to inoculate cell cultures as described in the inoculation procedures or up to a 1:5 dilution (1 part ovarian fluid to 4 parts anti-inc) (Section 2, 4.9.B “Antibiotic Incubation Medium (Anti-Inc) Made with HBSS for Sample Disinfection” or Section 2, 4.9.C “Antibiotic Incubation Medium (Anti-Inc) Made with Minimum Essential Medium (MEM-0) for Sample Disinfection”) may be made.
4. If a dilution is made, tubes are vortexed and incubated for two hours at 15°C or 12 to 24 hours at 4°C. Samples are then re-centrifuged at 2000-3000 X g and the supernatant is inoculated onto tissue culture plates as described in the inoculation procedures.