**Virus isolation and preservation**

**Tissue Pooling**

* Respiratory : Lung, trachea, air sacs (posterior)
* Digestive : Liver, pancreas, small intestine (duodenum, jejunum, ileum), cecum, proventriculus, ventriculus, large intestine
* Urinary – kidney
* Lymphoreticular system – spleen & Bursa of Fabricius
* Nervous – brain
* Cardiovascular – heart
* Reproductive – oviduct and ovaries

**Do’s of Tissue Processing**

* Do’s Do pool tissues from an organ system
* Digestive and nervous systems are processed separately
* Heart can be pooled with spleen and bursa
* Lung and spleen can be pooled
* Liver and kidney can be pooled

Keep tissues cold (on ice)

**Don'ts of Tissue Processing**

* Don’t pool tissues from more than one animal – Each animal should be separate

Antibody from one tissue can neutralize the virus from another tissue

* Don’t pool the brain with any other tissue
* Don’t pool digestive organ tissues with other organ system







**Processing Tissues for Isolation**

* Prepare 10% suspension in antibiotic diluent
* Centrifuge at 1,500 x g – 20 minutes
* Remove supernatant with pipette and place in sterile vial
* Incubate 1 hour at room temperature

**Processing Swab Pool Specimens**

* Swabs (tracheal and cloacal)
* Vortex (mix) to re-suspend material and rinse all swab material from the sides of the tubes
* Centrifuge 1,500 x g – 20 minutes
* Remove supernatant with pipette
* Add supernatant to antibiotic diluent (3-4 ml total)
* Incubate 1 hour at room temperature

**Antibiotics (final concentration)**

* Penicillin (10,000 IU/ml)
* Streptomycin (2,000 - 10,000 μg/ml)
* Gentamicin sulfate (1,000 μg/ml)
* Kanamycin sulfate (650 μg/ml)
* Amphotericin B (5-20 μg/ml)







**Preservation**

All viruses preserve in liquid nitrogen or -70C