Guideline for Sample Collection and Retention for Foreign Animal Disease Investigation

The objective of this document is to provide guidelines and recommendations for collection and preservation of diagnostic specimens to diagnose foot-and-mouth disease (FMD), classical swine fever (CSF), highly pathogenic avian Influenza (HPAI), Newcastle disease (NCD), vesicular stomatitis (VS) and bovine spongiform encephalopathy (BSE). Foreign animal disease investigations initiated by the foreign animal disease diagnostician (FADD) or NAHLN laboratory (from routine submission or surveillance) shall be conducted as outlined in the VS memo 580.4.

Information to be sent with samples:

- Name and address of owner where disease occurred with complete contact information
- Name and contact information of sample collector and/or submitter
- Referral control number assigned by the area veterinary in charge (AVIC) of the submitting State
- Disease suspected and test requested
- The species, breed, sex, age and identity of the animals sampled
- Date samples were collected and submitted
- List of samples submitted with type of transport media used
- Label each sample container with animal ID, type of sample, and date of collection
- Laboratory results, if submission is initiated by the NAHLN laboratory
- Case history:
    a. A list and description of the animals examined and findings of the clinical and post-mortem examination (with pictures if available)
    b. The length of time sick animals have been on the farm; if they are recent arrivals, from where did they originate
    c. The date of the first cases and of subsequent cases or losses, with any previous submission reference numbers
    d. A description of the spread of infection in the herd or flock
    e. The number of animals on the farm, the number of animals dead, the number showing clinical signs and their age, sex and breed

General guidelines for sample collection:

Animals to be sampled:

- Animals apparently normal in contact with sick animals
- Animals showing clinical signs representing the disease condition
- Terminally ill animals that can be sacrificed for necropsy or freshly dead animals

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Sample Collection:

Refer to sample collection chart to determine the type of samples to collect for specific diseases. Samples should be chilled immediately after collection and shipped on ice packs. The FADD or sample collector is encouraged to contact the laboratory for consultation on what samples to collect, if they have any questions regarding what samples to collect.

Transport Media:

- TBTB: tris buffered tryptose broth. Shelf life is 12 months at 4C
- BHI: brain heart infusion. Shelf life is 18 months at 4C
- DMEM: Dulbecco's Modified Eagle Medium for CSF nasal swab. Shelf life 12 is months at 4C

Sample Type:

➢ Live animals:

- Blood: Green, red and purple top tubes
- Swabs: Oral, nasal, lesions, tracheal and cloacal (birds) in transport media such as TBTB or BHI. Use Dacron swabs in 3 ml of media
- Skin scrapings, scabs, or epithelial flaps in sufficient volume of transport medium so that tissues are moist
- Tonsil scraping in 3 ml of transport media
- Vesicular fluid if available in equal amount of media
- Esophageal/pharyngeal (OP) fluid/probang sample when suspecting vesicular disease mainly FMD or VSV in large and small ruminants. Mix probang fluid with equal volume of TBTB media.

➢ Dead Animals:

- A complete necropsy should be performed and findings should be documented in a necropsy report
- A complete set of fresh tissues is preferred to collect, including but not limited to lymph nodes, spleen, tonsil, heart, liver, lung, kidney, brain (if neurological signs seen) and portions of intestine with lesion
- Another complete set of tissue should be collected and fixed in 10% neutral buffered formalin at a ratio of 1:10, tissue to formalin. Tissues should not be more than 1-1.5 cm thick to ensure quick fixation. Remember to sample all tissues with gross lesions including normal and abnormal areas. Collect the brain, when CNS signs are present
- Heart blood should be collected if blood can not be collected before death or euthanasia

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Specific guidelines and procedures for sample collection:

- Epithelial samples/vesicular fluid (FMD and other vesicular diseases):
    a. Ideally, at least 1 g of epithelial tissue should be collected from an unruptured or recently ruptured vesicle
    b. To avoid injury to personnel collecting the samples, as well as for welfare reasons, it is recommended that animals be sedated before samples are obtained
    c. When collecting vesicular material, the instruments and epithelium samples must not come in contact with disinfectant, as this will rapidly inactivate any virus that might be present
    d. Label each tube with date of collection, sample type and animal ID, and immediately place in a cooler containing ice packs

- Probang (for FMD and VSV):

  Where epithelial samples are not available from ruminant animals (in advanced or convalescent cases), or where infection is suspected in the absence of clinical signs (carrier animals), probang can be collected using a probang cup provided in the FADD kit.

  Procedures: ruminants
    a. Restrain the animal and open the mouth
    b. Pass the probang cup over the root of the tongue
    c. Place one hand over the pharyngeal area and continue to introduce the probang cup until it can be palpated in the upper part of the esophagus. Pass the probang cup backwards and forwards several [5-10] times, each time pushing far enough forward to push the cup into the cranial esophagus as evidenced by slight resistance to forward movement
    d. Gently withdraw the probang cup, keeping it upright to ensure that the OP fluid remains inside
    e. Visually inspect the material. Add equal volume of transport media (TBTB), the mixture should be shaken gently and should have pink to orange color as an indication of the neutral pH. Yellow color indicates unfavorable pH for the FMD virus to survive. Collected sample that is contaminated with ruminal contents will be acidic and must be discarded. Sampling should be repeated once the mouth of the animal has been rinsed with water or phosphate buffered saline. Samples seen to contain blood are not desirable but may be suitable for testing
    f. Label each tube with date of collection, sample type and animal ID, and immediately place in a cooler containing ice packs

- Tonsil scraping procedures (CSF suspect cases):
    a. For proper sampling, a sterile long-handled metal spoon such as an ice-tea spoon should be used
    b. Prop the mouth open using speculum and place the bowl of the sterile spoon past the hard palate down in to the upper throat. Long-handled spoon facilitates collection in market age or larger swine with longer palate. The palatine tonsil is reasonably easy to recognize because of the pitted appearance of the surface
    c. Gently scrape the bowl of the spoon over the surface of the tonsils in a back to front motion several times (2-3X). This will cause the tonsil to exude a mucosal excretion from the crypts (pits)
    d. On the third or fourth pass over the tonsil, the bowl of the spoon will collect a significant amount of sample, sometimes as much as 1 ml. It is important not to scrape too hard, as drawing blood is not desired
    e. Remove the spoon from the mouth taking care to avoid dragging the spoon full of sample across the hard palate
    f. Remove the sample from the spoon using a Dacron swab and place in a tube containing 3.0 ml of DMEM media
    g. Label each tube with date of collection, sample type and animal ID, and immediately place in a cooler containing ice packs

- Nasal swab procedures (CSF suspect cases):
    a. The pig should be properly restrained with the head positioned upward to allow easy access to the nasal cavity. Anesthesia is not needed
    b. Insert a sterile Dacron swab into the nasal cavity and gently swab the surface of the nasal mucosa with a circular and back and forth motion to cover as much as possible of the nasal mucosal surface. Avoid touching the skin as you enter the nasal cavity
    c. The swab will collect nasal mucosal secretions and surface epithelium. It is important not to scrape too hard, as drawing blood is not desired
    d. Remove the Dacron swab from one nostril and repeat the same procedure in the other nostril
    e. Place the Dacron swab with sample in a tube containing 3.0 ml of DMEM medium. Stir the nasal swab into the medium so that the sample is washed out from the swab into the medium
    f. Label each tube with date of collection, sample type and animal ID, and immediately place in a cooler containing ice packs

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- BSE tissue collection procedures:
    a. Remove the head at the atlanto-occipital joint.
    b. Using scissors and forceps open the dura mater and cut the cranial nerves, vessels, and connective tissue that connect the dura mater to the brainstem.
    c. Insert the brainspoon into the foramen magnum, severe the brainstem cranial to the obex region, and remove the brainstem including the obex region
    d. Place the brainstem and obex into a 50ml conical tube for shipping
    e. Label each tube with date of collection, sample type and animal ID, and immediately place in a cooler containing ice packs

- Cloacal swab procedures:
    a. Insert a sterile swab into the cloaca, swabbing the area thoroughly in order to collect epithelial cells from the cloacal lining. If collecting fecal swabs, insert the swab into freshly deposited wet feces in order to saturate the swab
    b. Place the swab into 3-4 ml of viral transport media and swirl vigorously to dispel the contents of the swab into the media
    c. Lift the swab out of the media and press the swab firmly against the side of the tube to remove any remaining liquid from the swab
    d. Discard the swab into a disinfectant solution
    e. Label each tube with date of collection, sample type and animal ID, and immediately place in a cooler containing ice packs

- Tracheal/oropharyngeal swab procedures:
    a. Insert a sterile swab into the oropharyngeal area, swabbing the area thoroughly in order to collect epithelial cells from the area. Swab the tracheal opening and draw the swab through the choanal cleft on the upper palate. The trachea may also be swabbed; however, the oropharyngeal area is easier to sample and can yield a higher titer
    b. Place the swab into 3-4 ml of viral transport media and swirl vigorously to dispel the contents of the swab into the media
    c. Lift the swab out of the media and press the swab firmly against the side of the tube to remove any remaining liquid from the swab
    d. Discard the swab into a disinfectant solution
    e. Label each tube with date of collection, sample type and animal ID, and immediately place in a cooler containing ice packs

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Samples to submit to NAHLN and NVSL:

- Two sets of diagnostic samples, outlined in the table below, may be sent simultaneously to the NAHLN laboratory for screening and to the appropriate NVSL laboratory for complete analysis and confirmation

- For complete diagnostic analysis and rule out of swine fever diseases (including CSF) and vesicular diseases (including FMD) at NVSL-FADDL, sufficient amount of samples, particularly vesicular tissues with minimum of 0.5 gram, are needed as multiple assays will be utilized

- In the event of insufficient amount of sample to collect, all samples should be submitted to the appropriate NVSL laboratory and not to the NAHLN laboratory

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Disease	Assay at NAHLN	Sample for NAHLN	Sample for NVSL (NVSL-FADDL or NVSL Ames)
Avian influenza	Realtime PCR	Cloacal swab, tracheal or oropharyngeal swab	Serum, cloacal swab, tracheal or oropharyngeal swab, fresh tissue; liver, spleen, kidney, lung and terminal intestine
Bovine spongiform encephalopathy	ELISA	Fresh brainstem at level of obex, in 50ml tube	The brainstem sent to the NAHLN lab will be forward to NVSL as needed
Classical swine fever	Realtime PCR	¼ tonsil	Serum, whole blood, ¾ tonsil, tonsil scraping, spleen, lymph node (mandibular and mesenteric), kidney, distal ileum
Foot-and-mouth disease	Realtime PCR	Vesicular tissue, oral swab, probang	Serum, whole blood, vesicular tissue, vesicular fluid, nasal and oral swab, scab, probang, lymph node
Newcastle disease	Realtime PCR	Cloacal swab, tracheal or oropharyngeal swab	Serum, cloacal swab, tracheal or oropharyngeal swab, fresh tissue; liver, spleen, kidney, lung and terminal intestine
Vesicular stomatitis	Complement fixation	Serum	Serum, whole blood, vesicular tissue, vesicular fluid, nasal and oral swab, scab, probang, lymph node

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Sample storage and retention time:

- Samples should be shipped immediately after collection, or stored at 4C and shipped no later than 3 days post collection

- The testing laboratory should retain the samples for a minimum of 30 days, at -20C for serum and at -70C for tissue, after the date of the final case report with the exception of high volume testing during a disease outbreak response or surveillance program

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DISEASE	SPECIMEN	MEDIUM	LAB
African Horse Sickness			
Equine (including Zebra)	Serum	Red Top Tube (10 ml)	FADDL
	Whole Blood	Green Top Tube (10 ml) Purple Top Tube (10 ml)	
	Fresh Tissue: Spleen, LN, Lung	Separate Whirlpak Bag Per Tissue Type	
	Set of Tissues	Formalin (10:1)	
African Swine Fever			
Swine	Serum	Red Top Tube (10ml)	FADDL
	Whole Blood	Green Top Tube (10 ml) Purple Top Tube (10 ml)	
	Fresh Tissue: Tonsil, Gastrohepatic LN, Renal LN, Spleen	Separate Whirlpak Bag Per Tissue Type	
	Set of Tissues	Formalin (10:1)	
Aino and Akabane[1]			
Bovine Ovine Caprine	Serum from fetus and dam	Red Top Tube (10 ml)	FADDL or NVSL - Ames
	Fresh tissue: Placenta, fetal muscle and nervous tissue	Separate Whirlpak Bag Per Tissue Type	
	Set of Tissues	Formalin (10:1)	
Avian Influenza – Highly Pathogenic			
Avian	Serum	Red Top Tube (2ml)	NVSL - Ames
	Tracheal Swab	Dacron Swab in BHI broth (3.5 ml) (can pool up to 5 swabs), do not pool tracheal and cloacal swabs together	
	Cloacal Swab	Dacron Swab in BHI broth (3.5 ml) (can pool up to 5 swabs), do not pool tracheal and cloacal swabs together	
	Fresh Tissue (liver, spleen, kidney, lung, terminal intestine)	For Each Bird: 1 whirlpak with intestine 1 whirlpak with pooled lung, liver, spleen and kidney	
Bluetongue[1] & Epizootic Hemorrhagic Disease[2]			
Bovine Ovine Caprine Cervid	Serum	Red Top Tube (10ml)	NVSL – Ames or FADDL (if vesicular lesions)
	Whole Blood from dam and newborn	Green Top Tube (10 ml) Purple Top Tube (10 ml)	
	Fresh tissue (dam): spleen, bone marrow	Separate Whirlpak Bags	
	Fresh tissue (newborn): spleen, lung, brain	Separate Whirlpak Bags	
	Set of Tissues	Formalin (10:1)	
Blue Eye Paramyxovirus			
Swine	Serum	Red Top Tube (10ml)	NVSL-Ames
	Whole Blood from dam and newborn	Green Top Tube (10 ml) Purple Top Tube (10 ml)	
	Nasal Swab	Dacron swab in TBTB (max 3 ml)	
	Fresh tissue (brain, spinal cord, lung, tonsil, spleen, reproductive tissues from sexually mature animals)	Separate Whirlpak bag per tissue type	
	Set of Fixed Tissues (brain, spinal cord, lung, tonsil, spleen)	Formalin (10:1)	

[1]Virus isolation, PCR and serology are done in Ames. PCR and serology are done at FADDL.
[2]Virus isolation, PCR and serology are done in Ames. Serology is done at FADDL.

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DISEASE	SPECIMEN	MEDIUM	LAB
Bovine Ephemeral Fever			
Bovine	Serum	Red Top Tube (10 ml)	FADDL
	Whole Blood	Green Top Tube (10 ml) Purple Top Tube (10 ml	
	Set of Tissues	Formalin (10:1)	
Bovine Spongiform Encephalopathy			
Bovine	Minimum for surveillance: Fresh Obex	50 ml tube or Whirlpak Bag	Contract Lab
	Complete FAD Investigation (after Rabies Tests) Fresh Obex and ½ Brain	Whirlpak Bag	NVSL - Ames
	½ Brain	Formalin (10:1)	
Classical Swine Fever			
Swine	Serum	Red Top Tube (10 ml)	FADDL
	Whole Blood	Green Top Tube (10 ml) Purple Top Tube (10 ml)	
	Dacron Swab: Nasal	TBTB (3 ml)	
	Tonsil Scraping (with Teaspoon)	TBTB (5 ml)	
	Fresh Tissue: Tonsil, Mandibular LN, Mesenteric LN, Spleen, Kidney, Distal Ileum	Separate Whirlpak Bag Per Tissue Type	
	Set of Tissues Including Brain	Formalin (10:1)	
Contagious Agalactia			
Ovine Caprine	Serum	Red Top Tube (10ml)	FADDL
	Dacron Swab: Nasal	BHI (3 ml)	
	Milk, Joint Fluid, Pleural Fluid, Pericardial Fluid	Sterile Tube or Red Top Tube	
	Udder & Associated LN, Lung	Separate Whirlpak Bags	
	Set of Tissues	Formalin (10:1)	
Contagious Caprine Pleuropneumonia			
Caprine	Serum	Red Top Tube (10ml)	FADDL
	Pleural Fluid	Sterile Tube or Red Top tube	
	Fresh tissue: Lung, Mediastinal LN	Separate Whirlpak Bags	
	Set of Tissues	Formalin (10:1)	
Contagious Bovine Pleuropneumonia			
Bovine	Serum	Red Top Tube (10 ml)	FADDL
	Pleural and Joint Fluid	Sterile Tube or Red Top Tube	
	Fresh tissue: Lung, Tracheobronchial LN and Mediastinal LN	Separate Whirlpak Bags	
	Dacron Swab: Nasal and Tracheobronchial	BHI (3ml)	
	Set of Tissues	Formalin (10:1)	

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DISEASE	SPECIMEN	MEDIUM	LAB
Foot and Mouth Disease			
Bovine Swine Camelids Cervid (including cloven-hoofed zoo animals & wildlife)	Serum	Red Top Tube (10 ml)	FADDL
	Whole Blood	Green Top Tube (10 ml) Purple Top Tube (10 ml)	
	Vesicular Tissue (vesicular epithelium – as large as practical)	Whirlpak Bags or TBTB (3 ml)	
	Vesicular Fluid	Sterile Tube or Red Top Tube (undiluted) or TBTB (50:50)	
	Dacron Swabs: Lesion, Nasal and Oral	TBTB (3 ml)	
	Esophogeal-Pharyngeal Fluid (Probang)	TBTB (50:50)	
	Crusts/Scabs	Tube or Whirlpak Bags, no media	
	Fresh Tissue (esp. Lymph Node)	Separate Whirlpak Bags	
	Set of Tissues	Formalin (10:1)	
Heartwater*			
Bovine Ovine Caprine Cervid	Serum	Red Top Tube (10 ml)	NVSL – Ames
	Whole Blood	Purple Top Tube (10 ml)	
	Brain Smear	Air dry before packaging. If receipt at lab to be > 24 hrs, fix with methanol before shipping. Note on paperwork if slide is fixed.	
	Fresh tissue (lymphoid tissues, brain, kidney	Separate Whirlpak Bag per tissue	
	Set of Tissues (lymphoid tissues, brain, kidney)	Formalin (10:1)	
Japanese Encephalitis			
Equine Swine	Serum	Red Top Tube (10 ml)	FADDL
	Whole Blood	Green Top Tube (10 ml) Purple Top Tube (10 ml)	
	Fresh tissue: Brain, Spinal Cord	Separate Whirlpak Bags	
	Set of Tissues	Formalin (10:1)	
Lumpy Skin Disease			
Bovine	Serum	Red Top Tube (10 ml)	FADDL
	Whole Blood	Green Top Tube (10 ml) Purple Top Tube (10 ml)	
	Skin Biopsy	TBTB (3 ml)	
	LN Aspirate	TBTB (3 ml)	
	Fresh Tissues: Skin, LN, Lung	Separate Whirlpak Bag Per Tissue Type	
	Set of Tissues	Formalin (10:1)	

*FADDL tests import cases from FMD-endemic countries.

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DISEASE	SPECIMEN	MEDIUM	LAB
Malignant Catarrhal Fever			
Bovine (Including wild ruminants)	Serum	Red Top Tube (10 ml)	NVSL – Ames or FADDL (if vesicular lesions)
	Whole Blood	Green Top Tube (10 ml) Purple Top Tube (10 ml)	
	Fresh Tissue: Spleen, lung, LN, Thyroid, Adrenal, Cornea, Liver & Kidney	Separate Whirlpak Bag Per Tissue Type	
	Set of Tissues	Formalin (10:1)	
Newcastle Disease - Exotic			
Avian	Serum	Red Top Tube (2ml)	NVSL-Ames
	Tracheal Swab	Dacron Swab in BHI broth (3.5 mo) (can pool up to 5 swabs), do not pool tracheal and cloacal swabs together	
	Cloacal swab	Dacron Swab in BHI broth (3.5 mo) (can pool up to 5 swabs), do not pool tracheal and cloacal swabs together	
	Fresh Tissue (liver, spleen, kidney, lung, terminal intestine)	For Each Bird: 1 whirlpak with intestine 1 whirlpak with pooled lung, liver, spleen and kidney	
Porcine Epidemic Diarrhea			
Swine	Serum	Red Top Tube (10 ml)	NVSL-Ames
	Fresh Tissue (feces, ileum, jejunum, duodenum, multiple sections of large intestine)	Separate Whirlpak bag per tissue type	
Peste des Petits Ruminants			
Ovine Caprine (Including wild small ruminants)	Serum	Red Top Tube (10 ml)	FADDL
	Whole blood	Green Top Tube (10 ml) Purple Top Tube (10 ml)	
	Dacron Swab: Nasal, Ocular, Oral, Fecal	TBTB (3 ml)	
	Fresh tissue: Bronchial LN, Mesenteric LN, Lung, Spleen, Intestinal Mucosa	Separate Whirlpak Bag Per Tissue Type	
	Set of Tissues	Formalin (10:1)	
Rabbit Hemorrhagic Disease			
Rabbit	Serum	Red Top Tube (5 ml)	FADDL
	Fresh Tissue: Liver, Spleen	Separate Whirlpak Bags	
	Set of Tissues	Formalin (10:1)	
Rift Valley Fever			
Ovine Bovine Caprine Camelid	Serum	Red Top Tube (10 ml)	FADDL
	Whole Blood	Green Top Tube (10 ml) Purple Top Tube (10 ml)	
	Fresh Tissue: Liver, Spleen, Brain, Placenta	Separate Whirlpak Bag Per Tissue Type	
	Set of Tissues	Formalin (10:1)	
Rinderpest			
Bovine Swine (including zoo animals & wildlife)	Serum	Red Top Tube (10 ml)	FADDL
	Whole Blood	Green Top Tube (10 ml) Purple Top Tube (10 ml)	
	Dacron Swabs: Lachrymal, Oral, Fecal	TBTB (3 ml)	
	Fresh Tissue: Oral lesions, Mesenteric LN, Prescapular LN, Spleen	Separate Whirlpak Bag Per Tissue Type	
	Set of Tissues	Formalin (10:1)	

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Sheep & Goat Pox			
Ovine Caprine	Serum	Red Top Tube (10 ml)	FADDL
	Whole Blood	Green Top Tube (10 ml) Purple Top Tube (10 ml)	
	Fresh Tissues: Skin, LN, Lung	Separate Whirlpak Bag	
	Skin Biopsy	TBTB (3 ml)	
	LN Aspirate	TBTB (3 ml)	
	Set of Tissues	Formalin (10:1)	
Swine Vesicular Disease			
Swine	Serum	Red Top Tube (10 ml)	FADDL
	Whole Blood	Green Top Tube (10 ml) Purple Top Tube (10 ml)	
	Vesicular Tissue (vesicular epithelium – as large as practical)	Tube or TBTB (3 ml)	
	Vesicular Fluid	Sterile Tube or Red Top Tube (undiluted) or TBTB (50:50)	
	Dacron Swab: Lesion, Nasal, Fecal	TBTB (3 ml)	
	Fresh Tissue: Skin, tongue and mucosa with lesions	Separate Whirlpak Bags	
	Set of Tissues	Formalin (10:1)	
Teschen/Talfan			
Swine	Serum	Red Top Tube (10 ml)	NVSL – Ames or FADDL (If CSF in differential)
	Fresh tissue (brain, spinal cord, lung, tonsil, spleen)	Separate Whirlpak bags per tissue	
	Set of Fixed tissues (brain, spinal cord, lung, tonsil, spleen)	Formalin (10:1)	
Venezuelan Equine Encephalomyelitis			
Equine	Serum	Red Top Tube (2 ml)	NVSL - Ames
	Whole Blood	Green Top Tube (2ml)	
	Brain, cerebrospinal fluid	Whirlpak bag (brain) and red-top tube (CSF)	
Vesicular Exanthema of Swine			
Swine	Serum	Red Top Tube (10 ml)	FADDL
	Whole Blood	Green Top tube (10 ml) Purple Top tube (10 ml)	
	Vesicular Tissue (vesicular epithelium – as large as practical)	Whirlpak Bags or TBTB (3 ml)	
	Vesicular Fluid	Sterile Tube or Red Top Tube (undiluted) or TBTB (50:50)	
	Dacron Swab: Lesion, Nasal, Oral	TBTB (3 ml)	
	Esophogeal-Pharyngeal Fluid (Probang)	TBTB (50:50)	
	Fresh Tissues: skin, tongue and mucosa with lesions	Separate Whirlpak Bags	
	Set of Tissues	Formalin (10:1)	

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Vesicular Stomatitis			
Bovine Swine Ovine Caprine Equine Camelid	Serum	Red Top Tube (10ml)	FADDL (Ruminant and Swine) & NVSL - Ames (Equine)
	Whole Blood (not for equine)	Green Top Tube (10 ml) Purple Top Tube (10 ml)	
	Vesicular Fluid	Sterile Tube or Red Top Tube (undiluted) or TBTB (50:50)	
	Vesicular Tissue (vesicular epithelium – as large as practical)	Whirlpak Bags or TBTB (max 3 ml)	
	Dacron Swab: Lesion, Nasal, Oral	TBTB (3 ml)	
	Esophogeal-pharyngeal Fluid (Probang) (Not required for equine)	TBTB (50:50)	
	Fresh Tissue: Skin, Tongue and Mucosa with lesions	Separate Whirlpak bags per tissue	
	Crusts/scabs	TBTB (enough to moisten scab/crust)	
	Set of Tissues (not for equine)	Formalin (10:1)	

*List of tissues to be placed in formalin – At a minimum, collect 1 cm thick sections of any lesion, trachea, esophagus, heart, lung, thoracic lymph nodes, liver, spleen, kidney, abdominal lymph nodes, bladder, stomach, duodenum, jejunum, ileum and colon. If neurologic signs, collect brain. If small lesion, put entire lesion in whirlpak for virology, NOT in formalin.

DO NOT FREEZE SAMPLES - Submit Cool on Frozen Gel Pacs