

# **TVM DL Syndromic Approach to Bovine Testing**

Necropsy Guidance



Version 1.0 (2021)

# TABLE OF CONTENTS

<b>Introduction to the Necropsy Guidance Documents .....</b>	<b>4</b>
How to Use the Guidance Documents.....	4
<b>How to Assemble a Practical Necropsy Field Kit.....</b>	<b>5</b>
<b>General Bovine Necropsy .....</b>	<b>6</b>
Necropsy Supplies .....	6
Important Submission History to Provide .....	6
Sample Collection & Packaging .....	6
Testing Options .....	11
<b>Bovine Respiratory Disease Necropsy.....</b>	<b>12</b>
Necropsy Supplies .....	12
Important Submission History to Provide .....	12
Sample Collection and Packaging .....	12
Testing Options – Available with the Samples Outlined in this Protocol .....	12
Testing Options - BRD Related Testing that Will Require Additional Samples .....	13
<b>Calf Diarrhea Necropsy.....</b>	<b>14</b>
Necropsy Supplies .....	14
Important Submission History to Provide .....	14
Sample Collection and Packaging .....	14
Testing Options - Available with the Samples Outlined in this Protocol .....	14
Testing Options - Calf Diarrhea Related Testing that will Require Additional Samples.....	15
<b>Bovine Adult Enteritis, Diarrhea, and/or Weight Loss Necropsy .....</b>	<b>16</b>
Necropsy Supplies .....	16
Important Submission History to Provide .....	16
Sample Collection and Packaging .....	16
Testing Options - Available with the Samples Outlined in this Protocol .....	17
Testing Options - Adult Cattle Gastrointestinal Disease Related Testing that will Require Additional Samples.....	17
<b>Bovine Abortion/Stillbirth/Perinatal Mortality Necropsy .....</b>	<b>19</b>
Necropsy Supplies .....	19
Important Submission History to Provide .....	19
Sample Collection and Packaging .....	19
Testing Options: Fetal Tissues - Available with the Samples Outlined in this Protocol .....	20
Testing Options: Dam Testing - Available with the Samples Outlined in this Protocol .....	21
Testing Options - Bovine Abortion Related Testing that will Require Additional Samples.....	21
<b>Bovine Neurologic Disease Necropsy .....</b>	<b>23</b>
Necropsy Supplies .....	23
Important Submission History to Provide .....	23
Sample Collection and Packaging .....	23
Testing Options - Available with the Samples Outlined in this Protocol .....	24
Testing Options - CNS Abnormality Related Testing that will Require Additional Samples.....	25
<b>Bovine Sudden Death Necropsy .....</b>	<b>26</b>
Necropsy Supplies .....	26
Important Submission History to Provide .....	26
Sample Collection and Packaging .....	26
Testing Options - Available with the Samples Outlined in this Protocol .....	27
Testing Options - Sudden Death Related Testing that will Require Additional Samples .....	28
<b>Appendix I - Necropsy Supply Guide.....</b>	<b>29</b>
<b>Appendix II - Sample Packaging and Shipment .....</b>	<b>32</b>
<b>Appendix III - Shipping Box Packing Guide .....</b>	<b>34</b>

<b>Contributors.....</b>	<b>35</b>
--------------------------	-----------

# Introduction to the Necropsy Guidance Documents

## How to Use the Guidance Documents

This section deals specifically with topics related to the performance of a necropsy in cattle suffering from key syndromic disease patterns. Additionally, on the front end, there is guidance related to creating a field necropsy kit and important considerations in the actual performance of a necropsy.

Most, if not all of us, spent a significant amount of time in school learning how to do a necropsy and as a part of that recognizing key gross lesions. Clinical skills and antemortem diagnostics (if performed) direct us to one or more of the key syndromic disease patterns. Most often, sampling is directed to a particular organ or organ system. But, in the case of “sudden death” or where the owner may not have observed the clinical syndrome or is unsure of it, the differential diagnosis list expands. A complete, thorough necropsy is usually required and sampling expands to be more inclusive to cover several possible conditions.

It is frustrating to get into the field to perform a necropsy and not have the essential tools and sample supplies needed to do a thorough job. In such situations, it may affect the quality of the sample, submission of the wrong sample, and/or inappropriate packaging. The end result may be a missed or inaccurate diagnosis.

This section provides the necessary information that will help direct you to choosing the correct samples for testing and how to preserve and package them to ensure their integrity.

Each disease syndrome is divided into subsections:

- Necropsy Supplies
- Important Submission History to Provide
- Sample Collection and Packaging
- Testing Options

Under each of these headings, syndrome-specific information is included to maximize the information obtained for directed sampling and testing. Please note the material included under the “Important Submission History to Provide” heading. This is critical information and, unfortunately, often excluded in submissions. It is also possible to include or submit digital images of key gross lesions if there is a question as to what they represent and/or their significance. TVMDL pathologists are available to help interpret and assess significance.

At the end of the section are two appendices, one providing a more concise listing of supplies and the second more specific instructions for packaging in preparation for shipping.

It is TVMDL’s hope that this section containing “Necropsy Guidance Documents” will be a real help in your practice in relation to those situations where an animal to be euthanized or is simply found dead and there is a need for necropsy and diagnostic work-up.

# How to Assemble a Practical Necropsy Field Kit



Complete Necropsy Kit and Sampling Supplies

Diagnosing animal diseases frequently requires a veterinarian or a technician to collect specimens in the field. This can be difficult without having the right tools on hand. For these occasions, the Texas A&M Veterinary Medical Diagnostic Laboratory suggests assembling a necropsy field kit. The kit should include the following tools and materials, all of which are available from a hardware store:

- A 15-inch crosscut saw with a coarse blade.
- Pruning loppers, 29 to 31 inches.
- A dead-blow mallet.
- A camping hatchet.
- A boning knife.
- One box of quart-size re-sealable bags.
- One box of gallon-size re-sealable bags.

In addition, include a set of surgical scissors and forceps along with personal protective equipment, such as gloves and eyewear. Also, wrap the tools with sharper edges in old towels to protect the other tools as well as your hands. The tools may be stored and carried in a military-style tactical bag or in a large duffle bag. The bag should be at least 32-inches long to contain the pruning loppers, and should including a shoulder strap for easier carrying. Suitable bags are available at sporting goods stores or online.



Basic Field Kit Hardware



Comprehensive Field Kit Hardware

# General Bovine Necropsy

The following is a guide to doing a complete necropsy when either a full work-up is needed or if there is no historical or gross information to help guide the diagnostic work-up before necropsy. TVMDL has syndrome specific necropsy guides available if a complete necropsy is not needed based on history and/or known risk factors.

## Necropsy Supplies

1. Digital photographs taken during the necropsy to document necropsy findings can be helpful when emailed to TVMDL Veterinary Diagnosticians or regulatory veterinarians if collaboration is needed after the necropsy. Using a ruler or other known frame of reference is helpful.
2. Cool packs if needed to keep samples cool in the field and during transport from the necropsy site to the clinic before shipment
3. Red and lavender top blood tubes for fluid samples and absorbent blood tube sleeves for shipping
4. Slides for blood film and cytology smears and slide containers for transport and shipment
5. Whirl-Pak bags or other sealable bags for fresh samples
6. Large specimen collection jars (150-250ml) with screw top lid, or appropriate number of smaller jars to be able to send 1 cup of rumen contents and possibly colon contents
7. Leak proof screw top jars (120 to 500 mL capacity) with formalin (60 to 250 mL) as needed to maintain a 10:1 formalin to tissue ratio
8. Permanent marker and/or labels for whirl-Pak bags, specimen cups, and formalin jars
9. Zip-close bag or plastic 10-12 mL ear notch container
10. Sandwich size Zip-close bag and TVMDL submission form
11. Secondary shipment bags x3: one gallon Zip-close bags, 95kPa large specimen transport bag, or similar bag with appropriate absorbent material
12. Appropriate sized insulated specimen shipment box, cool packs, packing tape
13. Dacron swabs, viral transport media swabs, aerobic culture swabs, and/or charcoal culture swabs if needed
14. Possible hardware needed: boning knife, sharpening steel, thumb forceps, several pairs of scissors, meat saw, hand axe or reciprocating saw, small wedge, mallet, rib cutters/pruning shears, eye protection, scalpel, needles, syringes, twine, ruler, pH strips

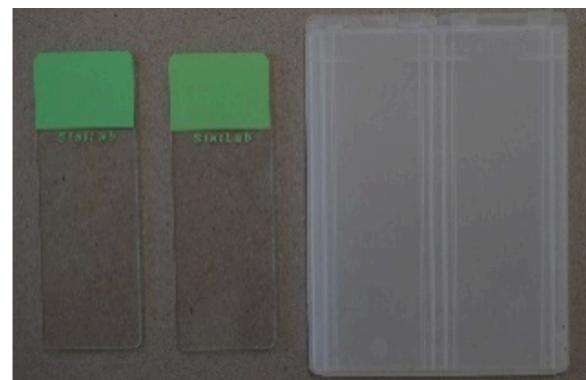
## Important Submission History to Provide

1. Any available treatment and vaccination history
2. Presenting clinical signs, physical exam findings, known disease progression, and impact at the herd level
3. Significant necropsy findings and suspicions, including preliminary differential diagnoses
4. Diagnostic goal: let us know how we can best help you and your client. What are your diagnostic needs?

## Sample Collection & Packaging

1. If there are any other questions about sample collection or test selection, please contact the TVMDL veterinary diagnosticians or pathologists at the College Station or Canyon laboratories for assistance before submission. If there is any suspicion of a reportable or foreign animal disease, contact the USDA area veterinarian in charge or the TAHC state veterinarian before removing any samples from the premise.
2. If it is possible to collect serum and blood samples from the animal before death, collect 6 to 10 mL of blood in a red top tube and remove from clot as soon as possible. Collect 4 to 6 mL

of EDTA blood in a lavender top tube. Prepare 2 blood film slides as soon as possible. Label tubes and slides with animal ID and sample type and keep cool before and during shipment to TVMDL. Slide containers for mailing and blood tube absorbent sleeves are available from TVMDL to help protect these items during shipment.



3. Please do not euthanize animals with indications of nervous system involvement by obliterating the skull/brain.
4. Sample collection (fresh and fixed) can focus on obvious lesions noted during the necropsy. The guidance below includes suggestions for tissue that is always valuable for diagnostics as well as suggestions for collection of abnormal tissue. A full complement of tissues and diagnostic samples should be collected when clinical history and gross necropsy findings do not indicate which systems are involved. When multiple animals are involved the samples should be collected, packaged, and identified separately if testing is to be done individually. If only a single animal is to be tested initially, pick the animal with the least postmortem change and indicate it clearly.
5. Before starting the necropsy, perform an external examination of the carcass and note any gross abnormalities in body condition, skin and hair coat, eyes, ears, feet, joints, external genitalia, body orifices, and mammary glands on the submission form. If external lesions are present, collect samples (fluid from vesicles, swabs, fixed and fresh tissue), note their origin location on the submission form, package them separate from the internal samples, and keep cool for shipping to TVMDL.



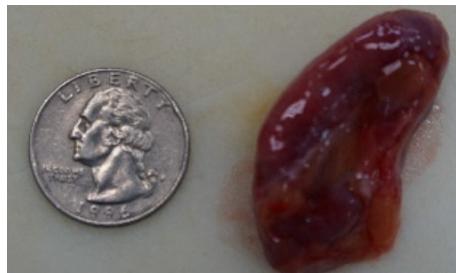
6. Collect a 3-5 mm ear notch sample and package in a small Zip-close bag or in a plastic ear notch tube, label with animal ID and keep cool for shipment to the lab.
7. Collect fresh samples of the following tissues, place cleanly in Whirl-Pak bags, label with animal ID and sample type, and keep cool before and during shipment to TVMDL.
  - a. Fresh tissues needed from: any diseased/abnormal tissue, representative and/or enlarged lymph nodes, trachea, normal lung, margin of diseased/normal lung if

appropriate, heart, each kidney (section that includes cortex and medulla), liver, spleen, ventral and dorsal rumen, duodenum, jejunum, ileum, cecum, spiral colon, descending colon

- b. If there are lesions noted in any of the organs, it may be useful to include the appropriate associated lymph nodes as well.



Bags for Samples



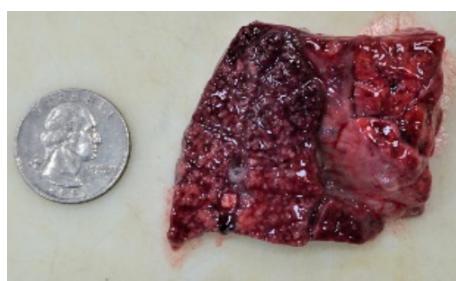
Lymph Node



Trachea



Trachea



Lung



Right and Left Ventricles



Kidney



Liver



Spleen



Rumen



Intestine



Intestine Loop

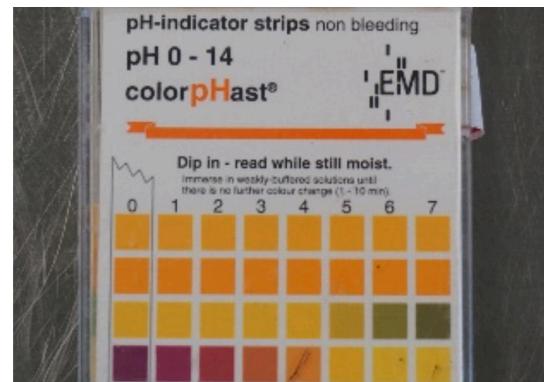
8. During the necropsy collect 3 mL of the following fluids in a sterile manner and transfer to a sterile red top tube. Label the tubes with animal ID and sample type and keep cool before and during shipment to TVMDL. If infection is suspected samples for cytologic examination can be similarly collected into lavender top tubes. Fresh slides are recommended as well.

- a. Always wanted: urine
- b. Include if the fluid is cloudy, unusual, blood-tinged, malodorous, or excessive: pleural fluid, pericardial fluid, abdominal effusion, joint effusion, bile, and/or heart blood (for culture)

9. Collect 1 cup of rumen contents into specimen collection cup or specimen bag, use tape to secure the top of the sample, label the specimen with animal ID and sample type and place in secondary specimen bag with absorbent for shipment to TVMDL.



Keep the rumen contents as cool as possible until arrival at TVMDL. Repeat this process on abomasal, gastroenteric, and/or colon contents if needed. If urea/ammonia intoxication is suspected, seal and freeze the rumen contents. If acidosis is suspected, use pH paper to measure rumen pH. Record pH measurement on submittal necropsy record. If microscopic analysis of rumen contents for toxic plants is needed, send rumen contents chilled, but not frozen, as soon as possible.



10. If microbiologic testing is desired on the gastrointestinal tract, collect 6-10 cm tied-off sections and package separate from other fresh samples in Whirl-Pak bags. Label the bags with animal ID and sample type, and keep cool before and during shipment to TVMDL. If more than one sample for culturing are packaged together, tie off the ends.



11. Collect approximately 1cm thick sections of the following tissues and fix in formalin at a ratio of 10:1 (formalin: sample). Seal the lid(s) of the formalin jar(s) with tape, label the jars with the animal ID and sample types and package in a secondary specimen bag with absorbent material, keeping the formalin away from the fresh samples and blood smear slides.

- a. Fixed samples needed from: enlarged or abnormal lymph nodes, representative lung

from dorsal and ventral fields (normal and abnormal sections as well as interface between good and bad lung), heart (right and left ventricular free wall as well as interventricular septum), each kidney (1 cm section that includes cortex and medulla), liver, dorsal and ventral rumen, abomasum, small intestine, cecum, spiral colon, descending colon, spleen, and diaphragm.



b. Additional fixed samples to send if indicated by history and/or necropsy findings: any tissue with lesions or lymph nodes that drain diseased tissue, nasal cavity/turbinates, trachea, thyroid glands, larynx, esophagus, pancreas, abnormal area of forestomachs, bladder, tongue, skeletal muscle, mammary glands, testes, uterus, ovaries, genitalia, bone marrow, abnormal bone, abnormal articular surfaces, and/or abnormal haired skin

12. The brain should be removed and examined in all cases with a history consistent with central nervous system abnormality and when the history, antemortem clinical signs, and necropsy do not identify another body system as the cause of death. If rabies is suspected, take appropriate precautions with personal protective equipment (gloves, goggles, sleeves). Remove the head and prepare for shipment if size and economics permit. Otherwise, remove brain from the skull and package fresh in such a way that it will not be crushed during shipment and ship chilled overnight to TVMDL. Clearly indicate if rabies is suspected on the submission form and the appropriate sample will be forwarded to TDSHS for testing before further testing is performed on the submission.

a. If the animal had signs of spinal cord involvement before death, dissect out the appropriate section of spinal cord (or cervical and thoracic spinal cord if location is not definitive), open the spinal column and inspect the surrounding tissues. Place 3 cm long sections of spinal cord and any other abnormal tissue within the spinal column in formalin for histopathology. If removal of the cord is not possible in the field, cross-section fragments of the vertebral column can be sent to the lab for spinal cord removal.

13. If testing on ocular fluid is indicated collect ocular fluid in a red top tube or submit the entire eye in a Whirl-Pak or Zip-close bag, label the tube or bag with the animal ID and specimen type and keep chilled for shipment to laboratory.



14. Package fresh specimens in large Zip-close or biohazard specimen shipping bag with absorbent, and keep them separate from fixed samples and rumen contents for shipping.
15. Seal jar(s) with rumen contents in large Zip-close or biohazard specimen shipping bag with absorbent, and keep separate from fresh and fixed samples for shipping.
16. Place all large secondary shipping bags containing samples (rumen contents, fresh, and fixed) and absorbent material in an insulated shipping box of appropriate size and integrity with cool packs and cushioning material. Make sure that the brain will not be crushed during shipment by the cool packs or other samples. Close the cooler portion of the insulated shipping box.
17. Place TVMDL submission form and any other paperwork needed in a separate Zip-close bag and place on top of shipping box cooler lid or in shipping box if insulated shipping box is not needed.
18. Close the shipping box and mark and label it appropriately for shipment. Detailed instructions of packaging and labeling submissions for shipment can be found on the Packaging Samples for Shipment page of the TVMDL website.

## **Testing Options**

Visit [tvmdl.tamu.edu](http://tvmdl.tamu.edu) or the TVMDL mobile app for a searchable listing of diagnostic tests available for bovine specimens. Bovine Syndromic Diagnostic Plans for respiratory disease, abortion, calf diarrhea, adult diarrhea and/or weight loss, sudden death, neurologic disease, keratoconjunctivitis, and biosecurity can be found on the TVMDL website.

Veterinary Diagnosticians are available at the College Station and Canyon laboratories for case consultation and assistance with test selection as well.

# Bovine Respiratory Disease Necropsy

## Necropsy Supplies

1. Whirl-pak bags for fresh samples (qty. 3-4), permanent marker and/or labels for whirl-pak bags
2. Formalin jars (120 mL) with formalin (60 mL)
3. If needed: culture swab with liquid or gel Amies media and charcoal culture swab with Amies media
4. Swabs for PCR testing must be Dacron or polyester (no cotton) with a plastic handle
5. Sandwich size Zip-close bag, TVMDL submission form, and Tape
6. Secondary shipment bags x3: gallon Zip-close bags, 95kPa large specimen transport bag, or similar bag
7. Appropriate sized insulated specimen shipment box, ice packs, packing tape

## Important Submission History to Provide

1. Treatment history – did the animal receive antibiotics or other drugs? How long before death? Was there any improvement with antibiotics or not?
2. Vaccination history or at least when was the last vaccination given and what was given?
3. What were the presenting clinical signs +/- physical exam findings?
4. Clinical onset, severity, and progression
5. Are other animals affected? What are the morbidity and mortality rates?

## Sample Collection and Packaging

1. Collect fresh samples from at least 3 different areas of the lungs, 4 cm section of the trachea, thoracic lymph nodes, and any other tissue that is indicated by the history or lesions found. Place the samples in separate whirl-pak (or similar) bags and label with animal ID and sample type. Keep samples chilled until shipment.
2. Collect 1 cm thick sections of lung (3), trachea (1), kidney (1), spleen (1), and any other tissues indicated by history or lesions and place in formalin jar with adequate formalin. Label jar with animal ID and sample types, tape lid, seal jar in large Zip-close or biohazard specimen shipping bag with absorbent, and keep separate from fresh samples for shipping.

## Testing Options – Available with the Samples Outlined in this Protocol

1. Respiratory Disease Panel – Bovine Viral (rtPCR) (IBR, PI3, BVDV, BRSV)
2. Bovine Coronavirus (rtPCR)
3. Bovine Influenza D Virus (rtPCR)
4. Respiratory Disease Panel – Bovine Bacterial (rtPCR) – (*Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Mycoplasma bovis*, *Trueperella pyogenes*)
5. Histopathology – Postmortem 8 or Fewer Tissues
6. Bacterial Identification – Livestock (Aerobic & Anaerobic Culture)
7. Antibiotic Susceptibility – Food Animal (Kirby-Bauer) or Antimicrobial Susceptibility – Food Animal (MIC), if pathogens are isolated on culture
8. Mycoplasma spp. – Livestock (Culture)
9. Anaplasma marginale (rtPCR)
10. Malignant Catarrhal Fever (rtPCR)

## **Testing Options - BRD Related Testing that Will Require Additional Samples**

Information on the following tests, including sample and shipping requirements can be found utilizing the searchable test catalog on the TVMDL website or in the TVMDL mobile app.

1. Hemoplasma (PCR) [Referral]
2. Bovine Viral Diarrhea Virus (Antigen Capture ELISA)
3. Bluetongue Virus (rTPCR)
4. Respiratory Disease Panel – Bovine Serology (MAT & VN) (BHV-1, PI3, BRSV, BVDV 1a/1b/2, *Mannheimia haemolytica*, *Histophilus somni*)
5. Bovine Adenovirus Type 3 (VN)
6. Cytology – Aspirate/Smear
7. Cytology – Body Fluid
8. Cytology (Digital)

Visit [tvmdl.tamu.edu](http://tvmdl.tamu.edu) or the TVMDL mobile app for a searchable listing of diagnostic tests available for bovine specimens. Bovine Syndromic Diagnostic Plans for respiratory disease, abortion, calf diarrhea, adult diarrhea and/or weight loss, sudden death, neurologic disease, keratoconjunctivitis, and biosecurity can be found on the TVMDL website.

Veterinary Diagnosticians are available at the College Station and Canyon laboratories for case consultation and assistance with test selection as well.

# Calf Diarrhea Necropsy

## Necropsy Supplies

1. Specimen collection cup with screw-top lid (4 oz or similar)
2. Sterile red top tube
3. Whirl-pak bags for fresh samples (qty. 3-4), permanent marker and/or labels for whirl-pak bags
4. Formalin jars (120 mL) with formalin (60 mL)
5. Sandwich size Zip-close bag, TVMDL submission form, and tape
6. Secondary shipment bags x2: gallon Zip-close bags, 95kPa large specimen transport bag, or similar bag
7. Appropriate sized insulated specimen shipment box, ice packs, packing tape

## Important Submission History to Provide

1. Treatment history – antibiotics, NSAIDs, antitoxins, fluids if given and when given in relation to death
2. Vaccination and colostrum history. Was the calf tested for adequate passive immunity? Did it receive colostrum/colostrum replacement/colostrum supplement from the cow, a bottle, esophageal feeder, etc.
3. Presenting clinical signs and significant physical exam findings
4. Clinical onset, severity, and progression. Were there any signs of systemic infection or septicemia? Was the diarrhea mucoid and/or bloody?
5. Are other animals affected? What are the morbidity and mortality rates?

## Sample Collection and Packaging

1. Collect 5-8 g of feces in a clean specimen collection cup, screw on lid, wipe contamination from outside of specimen cup, label with calf ID, and tape lid to cup. Keep sample chilled until shipment and arrival at TVMDL.
2. Collect Mesenteric lymph node and place in individual, labeled, whirl-pak (or similar) bag. Seal and keep chilled until shipment and arrival at TVMDL.
3. Collect 2 mL of intestinal contents in sterile red top tube and keep chilled.
4. Collect fresh intestinal samples from the affected area (distal jejunum, ileum, etc.) in whirl-pak (or similar) bags and label with calf ID and sample type. Keep samples chilled until shipment and arrival at TVMDL.
5. Collect 2-3 cm long sections of duodenum (1), jejunum (4), ileum (1), colon (2), and any other tissues indicated by history or lesions and place in formalin jar with adequate formalin. Label jar with calf ID and contents, tape lid, seal jar in large Zip-close or biohazard specimen shipping bag with absorbent, and keep separate from fresh samples for shipping.
6. Package feces, intestinal contents, and fresh specimens in large Zip-close or biohazard specimen shipping bag with absorbent, and keep separate from fixed samples for shipping.
7. Place both large secondary shipping bags containing samples (fresh and fixed) and absorbent material in an insulated shipping box of appropriate size and integrity with ice packs and cushioning material. Close the cooler portion of the insulated shipping box.
8. Place TVMDL submission form and any other paperwork needed in a separate Zip-close bag and place on top of shipping box cooler lid or in shipping box if insulated shipping box is not needed. Close the shipping box and mark and label it appropriately for shipment.

## Testing Options - Available with the Samples Outlined in this Protocol

Information on the following tests, including sample and shipping requirements can be found utilizing the searchable test catalog on the TVMDL website or in the TVMDL mobile app.

1. Calf Diarrhea Multiplex (rtPCR) (bovine coronavirus, bovine rotavirus group A, and cryptosporidium spp.)
2. Histopathology – Postmortem 8 or Fewer Tissues
  - a. Bovine Viral Diarrhea Virus – Persistently Infected (IHC) [Referral] if indicated
3. Bacterial Identification – Livestock (Aerobic & Anaerobic Culture) of feces
  - a. Including Salmonella spp. (Culture)
4. Salmonella Serotyping [Referral], if appropriate
5. E. coli Toxin Typing – Ruminant (rtPCR)
6. Parasite & Ova Identification (Fecal Floatation) – Qualitative
7. Parasite & Ova Count (McMasters)
8. Salmonella genus (rtPCR)
9. Clostridium perfringens Typing (rtPCR)
10. Cryptosporidium & Giardia Panel (rtPCR)
11. Electron Microscopy

## **Testing Options - Calf Diarrhea Related Testing that will Require Additional Samples**

Information on the following tests, including sample and shipping requirements can be found utilizing the searchable test catalog on the TVMDL website or in the TVMDL mobile app.

1. BVD PI – Bovine Viral Diarrhea Virus (Antigen Capture ELISA)
2. Milk replacer/post-pasteurization milk contamination
  - a. Mastitis (Culture)
  - b. Mycoplasma spp. – Milk (Culture)
  - c. Antimicrobial Susceptibility – Mastitis (MIC)
  - d. Antibiotic Susceptibility – Mastitis (Kirby-Bauer)

Visit [tvmdl.tamu.edu](http://tvmdl.tamu.edu) or the TVMDL mobile app for a searchable listing of diagnostic tests available for bovine specimens. Bovine Syndromic Diagnostic Plans for respiratory disease, abortion, calf diarrhea, adult diarrhea and/or weight loss, sudden death, neurologic disease, keratoconjunctivitis, and biosecurity can be found on the TVMDL website.

Veterinary Diagnosticians are available at the College Station and Canyon laboratories for case consultation and assistance with test selection as well.

# Bovine Adult Enteritis, Diarrhea, and/or Weight Loss Necropsy

## Necropsy Supplies

1. Specimen collection cup with screw-top lid (4 oz or similar) x2
2. Large specimen collection jar (300-500ml) with screw top lid, or appropriate number of smaller jars to be able to send a cup of rumen contents
3. Small Zip-close bag or plastic specimen tube
4. Whirl-pak bags for fresh samples (qty. 3-4), permanent marker and/or labels for whirl-pak bags
5. Formalin jars (120 mL) with formalin (60 mL)
6. Sandwich size Zip-close bag, TVMDL submission form, and Tape
7. Secondary shipment bags x3: gallon Zip-close bags, 95kPa large specimen transport bag, or similar bag
8. Appropriate sized insulated specimen shipment box, ice packs, packing tape

## Important Submission History to Provide

1. Number of animals affected (individual or several in a herd)? If several, what are the morbidity and mortality rates?
2. Is the primary clinical sign diarrhea, weight loss/wasting/lack of adequate growth, or both? Are there other significant clinical signs (down animals, fever, respiratory signs, etc.)?
3. Treatment history and how long treatment was before death.
4. Vaccination history or at least when and what was given at the last vaccination
5. Nutritional status, appetite, BCS and current nutrition being offered to animal(s)

## Sample Collection and Packaging

1. Collect 5-8 g of feces in 2 separate clean specimen collection cups, screw on lids, wipe contamination from outside of specimen cups, label with animal ID, and tape lid to cup. Keep sample chilled until shipment and arrival at TVMDL.
2. Collect 1 cup of rumen content from plant specimen dense area of the rumen, place in large specimen jar, screw on lid, wipe contamination from outside of specimen jar, label with animal ID, tape lid, and keep chilled.
3. Collect ≥3 mm ear notch and place in small Zip-close bag or plastic tube, label with animal ID, and keep chilled until shipment.
4. Collect fresh intestinal samples from the affected areas (distal jejunum, ileum, etc.), mesenteric lymph node, kidney, spleen, and ≥ 10 g liver and place in separate whirl-pak (or similar) bags and label with animal ID and sample type. Keep samples chilled until shipment.
5. Collect 2-3 cm long sections of duodenum (1), jejunum (4), ileum (1), colon (2), rectal tissue(1), mesenteric lymph node and/or other abnormal lymphoid tissue, liver(3), kidney(1 each), heart(1) and any other tissues indicated by history or lesions and place in formalin jar with adequate formalin. Label jar with animal ID and sample types, tape lid, seal jar in large Zip-close or biohazard specimen shipping bag with absorbent, and keep separate from fresh samples for shipping.
6. Seal jar(s) with rumen contents in large Zip-close or biohazard specimen shipping bag with absorbent, and keep separate from fresh and fixed samples for shipping.
7. Package feces and fresh specimens in large Zip-close or biohazard specimen shipping bag with absorbent, and keep separate from fixed samples and rumen contents for shipping.
8. Place all 3 large secondary shipping bags containing samples (rumen contents, fresh, and fixed) and absorbent material in an insulated shipping box of appropriate size and integrity with ice

- packs and cushioning material. Close the cooler portion of the insulated shipping box.
9. Place TVMDL submission form and any other paperwork needed in a separate Zip-close bag and place on top of shipping box cooler lid or in shipping box if insulated shipping box is not needed.
  10. Close the shipping box and mark and label it appropriately for shipment

## **Testing Options - Available with the Samples Outlined in this Protocol**

Information on the following tests, including sample and shipping requirements can be found utilizing the searchable test catalog on the TVMDL website or in the TVMDL mobile app.

1. Histopathology – Postmortem 8 or Fewer Tissues
  - a. Bovine Viral Diarrhea Virus – Persistently Infected (IHC) [Referral] if indicated
2. Bacterial Identification – Livestock (Aerobic & Anaerobic Culture) of feces
  - a. Including *Salmonella* spp. (Culture)
3. *Mycobacterium avium* subsp. paratuberculosis (rtPCR) for Johne's Disease
  - a. +/- *Mycobacterium avium* subsp. paratuberculosis (Solid Culture)
4. Bovine Leukemia Virus (rtPCR)
5. Bovine B4 Export Multiplex (rtPCR)
6. Bovine Viral Diarrhea Virus (Antigen Capture ELISA)
7. Toxicology Evaluation (Microscopy) of rumen content
8. Parasite & Ova Identification (Fecal Floatation) – Qualitative
9. Parasite & Ova Count (McMasters)
10. Calf Diarrhea Multiplex (rtPCR) (bovine coronavirus, bovine rotavirus group A, and cryptosporidium spp.)
11. *Salmonella* genus (rtPCR)
12. *Salmonella* Serotyping [Referral], if appropriate
13. *E. coli* Toxin Typing – Ruminant (rtPCR)
14. Metal & Mineral Panel (ICP/MS)
15. Trace Mineral Panel (ICP/MS)
16. *Leptospira* spp. (rtPCR)
17. *Anaplasma marginale* (rtPCR)
18. Malignant Catarrhal Fever (rtPCR)
19. pH (pH Electrode)
20. Electron Microscopy

## **Testing Options - Adult Cattle Gastrointestinal Disease Related Testing that will Require Additional Samples**

Information on the following tests, including sample and shipping requirements can be found utilizing the searchable test catalog on the TVMDL website or in the TVMDL mobile app.

1. *Mycobacterium avium* subsp. paratuberculosis – Serum or Plasma (ELISA) for Johne's Disease
2. *Salmonella Dublin* (ELISA)
3. Plant Identification
4. Toxicology Evaluation (Digital)
5. Cytology – Body Fluid
6. Cytology (Digital)
7. Drug Screen – Unknowns (LC/MS)
8. Parasite Identification (Gross Identification)
9. Sodium (FAAS) [Referral] on brain tissue
10. Herbicide (GC/MS)
11. Ionophores (LC/MS)
12. Cholinesterase (Kinetic pH/Kinetic Colorimetric)

Visit tvmdl.tamu.edu or the TVMDL mobile app for a searchable listing of diagnostic tests available for bovine specimens. Bovine Syndromic Diagnostic Plans for respiratory disease, abortion, calf diarrhea, adult diarrhea and/or weight loss, sudden death, neurologic disease, keratoconjunctivitis, and biosecurity can be found on the TVMDL website.

Veterinary Diagnosticians are available at the College Station and Canyon laboratories for case consultation and assistance with test selection as well.

# Bovine Abortion/Stillbirth/Perinatal Mortality

## Necropsy

### Necropsy Supplies

1. 5 sterile red top blood tubes and 1 sterile lavender top EDTA blood tube (more needed if herd serology is done)
2. At least 2 sterile 5-12 mL syringes and needles
3. Whirl-pak bags for fresh samples (qty. 5-8), permanent marker and/or labels for whirl-pak bags
4. At least 2 Formalin jars (120 mL) with formalin (60 mL)
5. Sandwich size Zip-close bag, TVMDL submission form, and Tape
6. Secondary shipment bags x4: gallon Zip-close bags, 95kPa large specimen transport bag, or similar bag
7. Appropriate sized insulated specimen shipment box, ice packs, packing tape

### Important Submission History to Provide

1. Estimated or known gestation length
2. Was the fetus a product of ET, AI, or live cover? Breed/genetics of the fetus
3. How many abortions have occurred and in what time frame? What percent of the brood herd has been affected? Heifers, cows, or both?
4. Are there any signs of systemic illness in the dams? Retained placenta?
5. Are there any gross abnormalities noted on the fetus or placenta?
6. Brood herd vaccination history
7. Open or closed herd?

### Sample Collection and Packaging

1. Collect 5 mL serum and 5 mL EDTA blood samples from the dam. Separate the clot from the serum, label with the ID of the dam, and keep cool for shipment to laboratory. Serologic herd profiles and screening are often more rewarding than individual animal serology for abortions. If abortion is a herd problem, serum samples from a representative population of affected and unaffected cows in the herd should be collected and sent with the fetal samples.
2. The entire fetus and placenta, chilled but not frozen, for necropsy at TVMDL is preferred when transportation to the laboratory is possible. If not, record any external abnormalities on the submission sheet and collect the samples below. Measure and record the crown rump length for estimation of gestational age if possible.
3. Describe any lesions identified on the placenta on the submission form if the placenta is available for examination.
4. After the abdomen and chest are opened, use a sterile syringe to collect 3-5 mL of thoracic fluid or fetal heart blood and transfer to sterile red top tube and label with fetus ID and sample type. Similarly collect 2-5 mL of stomach contents steriley and transferred to a sterile red top tube. Label with fetus ID and samples type. Describe any gross anatomic lesions found on necropsy on the submission form.
5. Collect fresh placenta, heart, thymus, lung, lymph node(s), liver, kidney, spleen, ear notch, and  $\frac{1}{2}$  the brain and put in sterile whirl-pak bags. If the brain is too friable to handle, the entire brain should be fixed. The lung, liver,  $\frac{1}{2}$  brain, and 2-3 pieces of cotyledonary placenta should be packaged individually in separate whirl-pak bags. Label the bags with fetus ID and sample type and keep chilled until shipment and arrival at TVMDL.
6. Fix the other  $\frac{1}{2}$  of the brain in formalin. Label jar with fetus ID and contents, tape lid, seal jar in large Zip-close or biohazard specimen shipping bag with absorbent, and keep separate from

- fresh samples for shipping.
7. Collect sections of tissue 1 cm thick from the following organs and place in formalin: placenta (5 sections – cotyledon if possible), thyroid gland, thymus, lung, heart, liver, spleen, lymph node(s), kidney, tongue, diaphragm, skeletal muscle ± adrenal glands. . Label jar with fetus ID and contents, tape lid, seal jar in large Zip-close or biohazard specimen shipping bag with absorbent, and keep separate from fresh samples for shipping.
  8. Collect an eyeball in a whirl-pak bag or ocular fluid in a sterile red top tube. Label with fetus ID and sample type.
  9. Package fresh specimens in large secondary Zip-close or biohazard specimen shipping bag with absorbent, and keep separate from fixed samples for shipping.
  10. Place all large secondary shipping bags containing samples (blood tubes, fresh and fixed tissues) and absorbent material in an insulated shipping box of appropriate size and integrity with ice packs and cushioning material. Close the cooler portion of the insulated shipping box.
  11. Place TVMDL submission form and any other paperwork needed in a separate Zip-close bag and place on top of shipping box cooler lid or in shipping box if insulated shipping box is not needed.
  12. Close the shipping box and mark and label it appropriately for shipment.

## **Testing Options: Fetal Tissues - Available with the Samples Outlined in this Protocol**

Information on the following tests, including sample and shipping requirements can be found utilizing the searchable test catalog on the TVMDL website or in the TVMDL mobile app.

1. Necropsy – Aborted Fetus or Fertile Egg
2. Histopathology – Postmortem 8 or Fewer Tissues
3. Abortion Panel – Livestock Bacterial (Culture)
4. Vitamin A (HPLC)
5. Trace Mineral Panel (ICP/MS)
6. Leptospira spp. (rtPCR)
7. Bovine Herpesvirus Type 1 (rtPCR)
8. Bovine Viral Diarrhea Virus (rtPCR)
9. Neospora caninum (rtPCR)
10. Respiratory Disease Panel – Bovine Viral (rtPCR) [BHV-1/BVDV/PI3/BRSV]
11. Bovine B4 Export Multiplex (rtPCR) [BHV-1/BVDV/BTV/BLV]
12. Anaplasma marginale (rtPCR)
13. Bunyavirus & Cache Valley Virus (PCR)
14. Mycoplasma spp. (PCR)
15. Mycoplasma bovis (rtPCR)
16. Respiratory Disease Panel – Bovine Bacterial (rtPCR) [*H. somni*, *M. bovis*, *M. haemolytica*, *P. multocida*, *T. pyogenes*]
17. Salmonella genus (rtPCR)
18. Bovine Viral Diarrhea Virus (Antigen Capture ELISA) – ear notch
19. Bovine Viral Diarrhea Virus – Persistently Infected (IHC) [Referral]
20. Virus Identification (IHC) [Referral]: BHV-1 IHC
21. Tritrichomonas foetus – Bovine (rtPCR)
22. Vitamin E (HPLC)
23. Nitrates – Fluids (Colorimetric)
24. Sodium (FAAS) [Referral] – Brain tissue
25. Brucella spp. (Culture)
26. Mycoplasma spp. – Livestock (Culture)/*Ureaplasma* culture
27. Salmonella spp. (Culture)

28. *Campylobacter* spp. (Culture)
29. *Campylobacter fetus* Differentiation (rtPCR)
30. *Campylobacter jejuni* (rtPCR)
31. Fungal Identification (Culture)
32. Virus Isolation (Cell Culture)
33. *Listeria monocytogenes* (PCR) [Referral]
34. Metal & Mineral Panel (ICP/MS)

## **Testing Options: Dam Testing - Available with the Samples Outlined in this Protocol**

Information on the following tests, including sample and shipping requirements can be found utilizing the searchable test catalog on the TVMDL website or in the TVMDL mobile app.

1. Abortion Panel – Bovine Serology Comprehensive
2. Abortion Panel – Bovine Serology Basic
3. Leptospira Panel – 5 Serovars (MAT)
4. Leptospira Panel – 6 Serovars (MAT)
5. Leptospira Panel – 7 Serovars (MAT)
6. Leptospira Panel – 8 Serovars (MAT)
7. *Neospora caninum* (ELISA)
8. *Brucella abortus* / *suis* (Card Agglutination)
9. *Brucella abortus* / *suis* (Plate Agglutination)
10. *Histophilus somni* (Microagglutination)
11. Bovine Herpesvirus Type 1 (VN)
12. Bovine Viral Diarrhea Virus Panel (VN) [1a/1b/2]
13. *Salmonella Dublin* (ELISA)
14. *Campylobacter fetus* (Microagglutination)
15. Cache Valley Virus (VN)
16. Bovine Viral Diarrhea Virus (Antigen Capture ELISA)
17. *Anaplasma marginale* (cELISA)
18. Bluetongue Virus (cELISA)
19. Bovine Leukemia Virus (ELISA)
20. Trace Mineral Panel (ICP/MS) – 0.5 mL EDTA blood + 2 mL serum or liver
21. Metal & Mineral Panel (ICP/MS) – 0.5 mL EDTA blood + 2 mL serum or liver
22. Copper (ICP/MS) – serum or liver
23. Selenium (ICP/MS) – EDTA blood or liver
24. Bovine Viral Diarrhea Virus (rtPCR)
25. Bovine B4 Export Multiplex (rtPCR) [BHV-1/BVDV/BTV/BLV]
26. Bluetongue Virus (rtPCR)
27. Epizootic Hemorrhagic Disease Virus (rtPCR)
28. Bluetongue & Epizootic Hemorrhagic Disease Virus Multiplex (rtPCR)
29. *Anaplasma marginale* (rtPCR)
30. Bovine Leukemia Virus (rtPCR)
31. Metabolic Profile – Transitional Herd

## **Testing Options - Bovine Abortion Related Testing that will Require Additional Samples**

Information on the following tests, including sample and shipping requirements can be found utilizing the searchable test catalog on the TVMDL website or in the TVMDL mobile app.

1. [Feed Nutritional Testing \[Referral\]](#)
2. [Mycotoxin \(LC/MS\) \[Referral\]](#)

3. Trichomonas – Bovine (Culture) – cervical wash
4. Campylobacter spp. (Culture) – cervical wash
5. Campylobacter fetus Differentiation (rtPCR) – cervical wash
6. Bull venereal disease testing
7. Nitrates – Hay/Forage (Ion Selective)
8. Vaginal/Uterine Cytology – Aspirate/Smear

Visit [tvmdl.tamu.edu](http://tvmdl.tamu.edu) or the TVMDL mobile app for a searchable listing of diagnostic tests available for bovine specimens. Bovine Syndromic Diagnostic Plans for respiratory disease, abortion, calf diarrhea, adult diarrhea and/or weight loss, sudden death, neurologic disease, keratoconjunctivitis, and biosecurity can be found on the TVMDL website.

Veterinary Diagnosticians are available at the College Station and Canyon laboratories for case consultation and assistance with test selection as well.

# Bovine Neurologic Disease Necropsy

## Necropsy Supplies

1. 2x Specimen collection cup with screw-top lid(4 oz or similar)
2. 1-2x Sterile red top tube
3. Whirl-pak bags for fresh samples (qty. 5-8), permanent marker and/or labels for whirl-pak bags
4. Formalin jars (120 mL) with formalin (60 mL)
5. 2x Large specimen collection jars (300-500ml) with screw top lid, or appropriate number of smaller jars to be able to send 2 cups of rumen contents
6. Zip-close bags for eye, feed, and forage if needed
7. If needed: culture swab with Amies media and charcoal culture swab with Amies media
  - a. Swabs for PCR testing must be Dacron or polyester (no cotton) with a plastic handle
8. Sandwich size Zip-close bag, TVMDL submission form, and Tape
9. Secondary shipment bags x4: gallon Zip-close bags, 95kPa large specimen transport bag, or similar bag
10. Appropriate sized insulated specimen shipment box, ice packs, packing tape

## Important Submission History to Provide

1. Treatment history -- did the animal receive antibiotics, steroids, or other drugs? How long before death? Was there any improvement with treatment or not?
2. Presenting signs, severity, and progression. Was the animal down for any amount of time before death? Aggressive? Blind? Tremoring? Seizures?
3. Vaccination history or at least when the last vaccination was and what was used.
4. Are other animals affected? What are the morbidity and mortality rates?
5. Diet
6. Is this a rabies suspect?

## Sample Collection and Packaging

1. If Rabies virus is suspected, take appropriate precautions with personal protective equipment (gloves, goggles, sleeves). Remove the brain from the skull and prepare for shipment or remove brain from the skull and package fresh so that it will not be crushed during shipment.
2. Perform gross necropsy. Collect fresh samples from any affected areas, lung, kidney, spleen, and  $\geq 10$  g liver and place in separate whirl-pak (or similar) bags and label with animal ID and sample type. Keep samples chilled until shipment.
3. Collect 5-8 g of feces in a clean specimen collection cup, screw on lid, wipe contamination from outside of specimen cup, label with calf ID, and tape lid to cup. Keep sample chilled until shipment and arrival at TVMDL.
4. Collect 2 cups of rumen content from plant specimen dense area of the rumen, place in large specimen jar, screw on lid, wipe contamination from outside of specimen jar, label with animal ID, tape lid, and keep 1 chilled and freeze the other and keep frozen for shipment to the laboratory.
5. Collect 1 cm thick sections of sublumbar lymph nodes and/or other abnormal lymphoid tissue, liver(3), kidney(1 each), lung(2), heart(1), and any other tissues indicated by history or lesions and place in formalin jar with adequate formalin. Label jar with animal ID and sample types, tape lid, seal jar in large Zip-close or biohazard specimen shipping bag with absorbent, and keep separate from fresh samples for shipping.
6. If the animal had signs of spinal cord involvement before death, dissect out the appropriate section of spinal cord, open the spinal column and inspect the surrounding tissues. Place 2-3

- cm long sections of spinal cord and any other abnormal samples in formalin for histopathology.
7. Collect ocular fluid in a red top tube or collect and package the entire eye in a bag, label the tube or bag with the animal ID and specimen type and keep chilled for shipment to laboratory.
  8. Package fresh specimens in large Zip-close or biohazard specimen shipping bag with absorbent, and keep separate from fixed samples and rumen contents for shipping.
  9. Seal jar(s) with rumen contents in large Zip-close or biohazard specimen shipping bag with absorbent, and keep separate from fresh and fixed samples for shipping.
  10. Place all large secondary shipping bags containing samples (rumen contents, fresh, and fixed) and absorbent material in an insulated shipping box of appropriate size and integrity with ice packs and cushioning material. Make sure that the brain will not be crushed during shipment by the ice packs or other samples. Close the cooler portion of the insulated shipping box.
  11. Place TVMDL submission form and any other paperwork needed in a separate Zip-close bag and place on top of shipping box cooler lid or in shipping box if insulated shipping box is not needed.
  12. Close the shipping box and mark and label it appropriately for shipment.

## **Testing Options - Available with the Samples Outlined in this Protocol**

Information on the following tests, including sample and shipping requirements can be found utilizing the searchable test catalog on the TVMDL website or in the TVMDL mobile app.

1. Rabies Virus (FA) [Referral] – fresh brain
2. Histopathology – Postmortem 8 or Fewer Tissues
3. Histopathology – Postmortem 9 or More Tissues
4. Bacterial Identification – Livestock (Aerobic & Anaerobic Culture)
5. Listeria monocytogenes (PCR) [Referral]
6. Sodium (FAAS) [Referral] – Fresh Brain
7. Calcium – ocular fluid
8. Magnesium – ocular fluid
9. Vitamin A (HPLC) – Liver
10. Vitamin E (HPLC) – Liver
11. Lead (ICP/MS) – Kidney
12. Bovine Herpesvirus Type 1 (rtPCR)
13. Respiratory Disease Panel – Bovine Bacterial (rtPCR) [*H. somni*, *M. bovis*, *M. haemolytica*, *P. multocida*, *T. pyogenes*]
14. Anaplasma marginale (rtPCR)
15. Ammonia (Distillation) – Frozen Rumen Contents
16. Malignant Catarrhal Fever (rtPCR)
17. Botulism ABC rtPCR [Referral]
18. Hemoparasite Examination (Wright-Giemsa Stain)
19. Babesia bigemina (CF) [Referral]
20. Babesia bovis (CF) [Referral]
21. Virus Isolation (Cell Culture)
22. Ionophores (LC/MS) - Rumen content
23. Cholinesterase (Kinetic pH/Kinetic Colorimetric) – fresh brain or EDTA blood
24. Parasite & Ova Identification (Fecal Floatation)
25. Parasite & Ova Count (Wisconsin)
26. Parasite & Ova Count (McMasters)
27. Bacterial Identification – Livestock (Aerobic & Anaerobic Culture)
28. Cytology – Aspirate/Smear
29. Cytology (Digital)
30. Cytology – Body Fluid: CSF

- 31. Microprotein - CSF
- 32. Bovine Leukemia Virus (ELISA)
- 33. Bovine Leukemia Virus (rtPCR)
- 34. Bovine B4 Export Multiplex (rtPCR) [BVD/BLV/BHV1/BTV]
- 35. Toxicology Evaluation (Microscopy) – rumen content
- 36. Toxicology Evaluation (Digital)
- 37. Neospora caninum (rtPCR) – brain
- 38. Salmonella genus (rtPCR)
- 39. Respiratory Disease Panel – Bovine Viral (rtPCR) [BVD/BHV1/PI3/BRSV]
- 40. Bluetongue Virus (rtPCR)
- 41. Bluetongue Virus Serotyping (rtPCR)
- 42. Bluetongue Virus Isolation (Egg Inoculation)
- 43. Bluetongue & Epizootic Hemorrhagic Disease Virus Multiplex (rtPCR)
- 44. Clostridium perfringens Typing (rtPCR)
- 45. Trace Mineral Panel (ICP/MS)
- 46. Metal & Mineral Panel (ICP/MS)
- 47. Drug Screen – Unknowns (LC/MS)
- 48. Pesticides Screen (GC/MS)
- 49. Carbamate Insecticides (LC/MS)
- 50. Herbicide (GC/MS)

### **Testing Options - CNS Abnormality Related Testing that will Require Additional Samples**

Information on the following tests, including sample and shipping requirements can be found utilizing the searchable test catalog on the TVMDL website or in the TVMDL mobile app.

- 1. Toxicology Evaluation (Microscopy) – Blue Green Algae
- 2. Mycotoxin (LC/MS) [Referral], Aflatoxin (ELISA), Fumonisin (ELISA)
- 3. Ionophores (LC/MS)
- 4. Feed Nutritional Testing [Referral]
- 5. Mastitis (Culture), Mycoplasma spp. – Milk (Culture)

Visit [tvmdl.tamu.edu](http://tvmdl.tamu.edu) or the TVMDL mobile app for a searchable listing of diagnostic tests available for bovine specimens. Bovine Syndromic Diagnostic Plans for respiratory disease, abortion, calf diarrhea, adult diarrhea and/or weight loss, sudden death, neurologic disease, keratoconjunctivitis, and biosecurity can be found on the TVMDL website.

Veterinary Diagnosticians are available at the College Station and Canyon laboratories for case consultation and assistance with test selection as well.

# Bovine Sudden Death Necropsy

## Necropsy Supplies

1. 2x Specimen collection cup with screw-top lid(4 oz or similar)
2. 1-2x Sterile red top tube
3. Whirl-pak bags for fresh samples (qty. 5-8), permanent marker and/or labels for whirl-pak bags
4. Formalin jars (120 mL) with formalin (60 mL)
5. 2x Large specimen collection jars (300-500ml) with screw top lid, or appropriate number of smaller jars to be able to send 2 cups of rumen contents
6. Zip-close bags for eye, feed, and forage if needed
7. If needed: culture swab with Amies media and charcoal culture swab with Amies media
  - a. Swabs for PCR testing must be Dacron or polyester (no cotton) with a plastic handle
8. Sandwich size Zip-close bag, TVMDL submission form, and tape
9. Secondary shipment bags x4: gallon Zip-close bags, 95kPa large specimen transport bag, or similar bag
10. Appropriate sized insulated specimen shipment box, ice packs, packing tape

## Important Submission History to Provide

1. Estimated time of death and time of necropsy
2. Vaccination history or at least when was the last vaccination given and what was given
3. Any significant observational or gross findings
4. Any recent changes in management, diet, or environment. Owner suspicions. Veterinary impressions.
5. Were there any clinical signs before death?
6. Are other animals affected? Over what time period? What is the mortality rate?
7. What toxic plants or toxins, if any, could the animal have been exposed to?

## Sample Collection and Packaging

1. If Anthrax is suspected and the risks associated with a full necropsy are unacceptable, draw blood from the jugular into a red top tube, identify and keep chilled for shipment. If full necropsy is desired, complete the additional steps.
2. Collect 1 cm thick sections of heart, lung, spleen, liver, kidney, and any other tissues indicated by history or lesions and place in formalin jar with adequate formalin. Label jar with animal ID and sample types, tape lid, seal jar in large Zip-close or biohazard specimen shipping bag with absorbent, and keep separate from fresh samples for shipping.
3. Collect fresh skeletal muscle, heart, lung, spleen, intestinal samples, mesenteric lymph node, sublumbar lymph node, kidney, and  $\geq 10$  g liver and place in separate whirl-pak (or similar) bags and label with animal ID and sample type. Keep samples chilled until shipment.
4. Collect 2 cups of rumen content from plant specimen dense area of the rumen, place in large specimen jar, screw on lid, wipe contamination from outside of specimen jar, label with animal ID, tape lid, and keep 1 chilled and freeze the other and keep frozen for shipment to the laboratory.
5. Collect  $> 10$  mL of urine in a clean specimen collection cup, screw on lid, wipe contamination from outside of specimen cup, label with animal ID, and tape lid to cup. Keep sample chilled until shipment and arrival at TVMDL.
6. Collect ocular fluid in an red top tube or collect and package the entire eye in a bag, label the tube or bag with the animal ID And specimen type and keep chilled for shipment to laboratory.
7. Open skull and carefully remove and inspect brain and meninges. Package and send the entire brain to lab fresh, keep chilled, package so that the brain is protected from crushing during

- shipment. Please get the brain to the lab as soon as possible. If history or lesions indicate that spinal cord samples need to be evaluated, send fresh spinal cord with the brain and fix several sections in formalin before shipment as well.
8. Collect 20-50 mL of water, if available, in a specimen collection cup, screw on lid, wipe contamination from outside of specimen cup, label with animal ID and specimen type, and tape lid to cup. Keep sample chilled until shipment and arrival at TVMDL.
  9. Collect 1-2 pounds of feed and or hay, if available, in a large Zip-close bag and keep chilled until shipment to the lab.
  10. Seal jar(s) with rumen contents in large Zip-close or biohazard specimen shipping bag with absorbent, and keep separate from fresh and fixed samples for shipping.
  11. Package water, feed, and fresh specimens in large Zip-close or biohazard specimen shipping bag with absorbent, and keep separate from fixed samples and rumen contents for shipping.
  12. Place all large secondary shipping bags containing samples (rumen contents, fresh, and fixed) and absorbent material in an insulated shipping box of appropriate size and integrity with ice packs and cushioning material. Make sure that the brain will not be crushed during shipment by the ice packs or other samples. Close the cooler portion of the insulated shipping box.
  13. Place TVMDL submission form and any other paperwork needed in a separate Zip-close bag and place on top of shipping box cooler lid or in shipping box if insulated shipping box is not needed.
  14. Close the shipping box and mark and label it appropriately for shipment.

## **Testing Options - Available with the Samples Outlined in this Protocol**

Information on the following tests, including sample and shipping requirements can be found utilizing the searchable test catalog on the TVMDL website or in the TVMDL mobile app.

1. Histopathology – Postmortem 8 or Fewer Tissues
2. Histopathology – Postmortem 9 or More Tissues
3. Sodium (FAAS) [Referral] – Fresh Brain
4. Bacterial Identification – Livestock (Aerobic & Anaerobic Culture)
5. Bacillus anthracis (Culture)
6. Clostridium spp. (FA)
7. Nitrates – Fluids (Colorimetric) – ocular fluid
8. Toxicology Evaluation (Microscopy) – rumen content
9. Cyanide (Colorimetric) – frozen rumen contents
10. Ammonia (Distillation) – frozen rumen content
11. Toxicology Evaluation (Digital)
12. Anaplasma marginale (rtPCR)
13. Lead (ICP/MS) – kidney
14. Heavy Metal Panel (ICP/MS) – liver
15. Arsenic – Inorganic (Reinsch) – rumen content
16. Arsenic (ICP/MS) – liver
17. Cholinesterase (Kinetic pH/Kinetic Colorimetric) – fresh brain
18. Copper (ICP/MS) – kidney, urine, feed
19. Selenium (ICP/MS) – liver, feed
20. Leptospira spp. (rtPCR)
21. Malignant Catarrhal Fever (rtPCR)
22. Petroleum (GC/MS)
23. Drug Screen – Unknowns (LC/MS)
24. Pesticides Screen (GC/MS)
25. Carbamate Insecticides (LC/MS)
26. Herbicide (GC/MS)

27. Anticoagulant Rodenticides (LC/MS)
28. pH (pH Electrode) – rumen content
29. Chloride (Colorimetric) – rumen content
30. Metal & Mineral Panel (ICP/MS) – liver
31. Water Analysis – Routine [Referral]
32. Water Analysis – Routine & Heavy Metals [Referral]
33. Water Analysis – Routine & Metals [Referral]
34. Chloride (Colorimetric) – Water
35. Petroleum (GC/MS) – Water
36. Nitrates – Fluids (Colorimetric) – Water
37. Feed Nutritional Testing [Referral]
38. Protein – Hay or Forage [Referral]

### **Testing Options - Sudden Death Related Testing that will Require Additional Samples**

Information on the following tests, including sample and shipping requirements can be found utilizing the searchable test catalog on the TVMDL website or in the TVMDL mobile app.

1. Nitrates – Hay/Forage (Ion Selective)
2. Cyanide (Colorimetric) – Fresh Forage
3. Arsenic – Inorganic (Reinsch) – soil
4. Plant Identification

Visit [tvmdl.tamu.edu](http://tvmdl.tamu.edu) or the TVMDL mobile app for a searchable listing of diagnostic tests available for bovine specimens. Bovine Syndromic Diagnostic Plans for respiratory disease, abortion, calf diarrhea, adult diarrhea and/or weight loss, sudden death, neurologic disease, keratoconjunctivitis, and biosecurity can be found on the TVMDL website.

Veterinary Diagnosticians are available at the College Station and Canyon laboratories for case consultation and assistance with test selection as well.

# Appendix I - Necropsy Supply Guide



Complete Necropsy Kit: Hardware and Sampling Supplies



Necropsy Kit Hardware and Procedure Supplies



Necropsy Kit Diagnostic Sampling and Packaging Supplies

## Necropsy Hardware and Procedure Supplies

---



Necropsy Knives



Sharpening Steel



Meat Saw / Hand Saw



Rib Cutters / Pruning Shears



Cleaver / Wedge and Mallet



Sharp Scissors



Thumb Forceps



Syringe and Needles



Scalpel Blades and Handle



Ruler



Twine



Eye Protection

## Necropsy Diagnostic Sampling and Packaging Supplies



Camera



Cold Packs and Cooler



Whirl-Pak or Zip-Close Bags



Specimen Collection Jars



Specimen Collection Jars



Jars with 10% NB Formalin



Dacron Swabs and Collection Tube



Bacteriology Swabs and Transport Gel



Plastic Tube or Collection Tube for Ear Notch



Sample Collection Tubes and Sleeves



Glass Slides and Slide Transporter



Aluminum Foil and Collection Tube



95kPa and Zip-Close Transport Bags



Submission Form and Zip-Close Bag



Cold Packs, Shipping Container, Packing Tape

# Appendix II - Sample Packaging and Shipment

There are four steps to safely packaging a sample for shipping to TVMDL:

## Step 1. Classify Your Sample

- Category A infectious substance

Infectious substance in a form that, when exposure to it occurs, is capable of causing permanent disability, life threatening, or fatal disease in otherwise healthy humans or animals (IATA Dangerous Goods Regulations §3.6.2.2.2.1).

*If you suspect you have a Category A infectious substance, please contact us at 979.845.3414 or toll free at 1.888.646.5623.*

- Category B infectious substance

An infectious substance that is transported in a form that, when exposure to it occurs, is NOT capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. TVMDL recommends all suspect infectious disease shipments be classified and shipped as a Biological Substance Category B.

- Exempt animal specimen

Specimen collected directly from animals for research, diagnosis, disease treatment and prevention which there is a minimal likelihood that pathogens are present.

Examples include: chemistry panel, CBC, endocrine testing, surveillance serology, export or movement testing, and vaccine titer.

## Step 2. Package Your Sample

- The packaging must consist of three components

- primary receptacle
- secondary packaging
- rigid outer packaging

- **Primary receptacles** must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary packaging.

- **Secondary packaging** must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

- A TVMDL submission form with appropriate client information, patient information, an itemized list of contents, and tests requested should be enclosed between the secondary and outer packaging.

- At least one surface of the **outer packaging** must have a minimum dimension of 100 mm x 100 mm (4 inches x 4 inches).

- Cold packs, dry ice or other refrigerant, if needed, may be placed around the secondary

packaging, but must not compromise the paperwork or inner/outer containers upon melting, sweating, etc.

- NOTE: dry ice requires a Class 9 label (UN number 1845; PSN: dry ice or carbon dioxide solid) and the weight of the dry ice in kg form.

#### For Liquid Substances

- The primary receptacle is leak-proof
- The secondary packaging is leak-proof
- Absorbent material is in sufficient quantity to absorb the entire contents placed between the primary and secondary packaging

#### For Solid Substances

- The primary receptacle is sift-proof
- The secondary receptacle is sift-proof
- If multiple fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated so as to prevent contact between them.

#### Step 3. Mark or Label Your Package

- The outer packaging must have the following information:
  - The name, address and telephone number of a responsible person on the waybill or on the package.
  - The shipper's and consignee's addresses.
  - For "Biological Substance, Category B", this label must be present in letters at least 6 mm high and must be marked on the outer package adjacent to a diamond-shaped UN3373 label.
  - For "Exempt Animal Specimen", this label must be present on the outer package in letters at least 6 mm high. NOTE: this classification still requires proper packaging (see Step 2 above).
  - Include a Class 9 label, including UN1845 label, and net weight if packaged with dry ice.

#### Step 4. Document Your Shipment

- A Shipper's Declaration is NOT required.
- Air Waybill — If applicable, the text "Biological Substance, Category B" and "UN 3373" should be documented on the waybill.
- If shipping with dry ice, UN 1845 and net weight of dry ice must be indicated.

Laboratory shipping addresses can be found at [tvmdl.tamu.edu](http://tvmdl.tamu.edu) or via the TVMDL app.

# Appendix III - Shipping Box Packing Guide



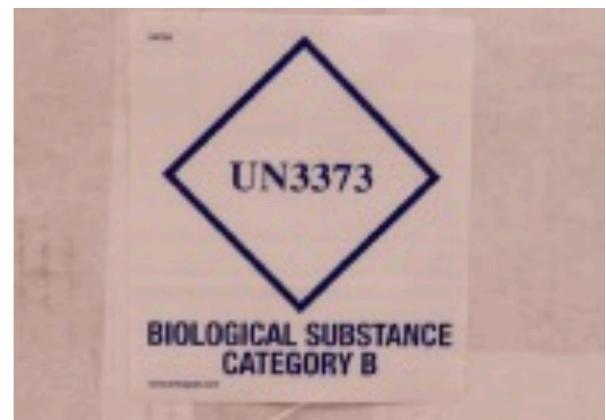
**Step 1:** Prepare a rigid outer package that will contain the secondary packaging. Use a cold pack if specimen must remain at a refrigerated temperature.



**Step 2:** To prevent damage to specimens in transit, ensure blood tubes have adequate cushioning and are bagged to prevent leakage. Add a layer of filler between the cold packs and specimen. Package paperwork in its own bag.



**Step 3:** Add additional filler to decrease empty within the secondary packaging. Secure the entire package with tape.



**Step 4:** Label the package in accordance to the specimen's classification.

# Contributors

1. **William Gilmore, DVM, PhD**  
Pathologist, TVMDL-Canyon, Retired
  - General Necropsy, reviewer
2. **Gabriel Gomez, DVM, PhD, DACVP**  
Section Head, Anatomic Pathology, TVMDL-College Station
  - General Necropsy, reviewer
3. **Gayman Helman, DVM, PhD, MA, DACVP**  
Resident Director, TVMDL-Canyon, Retired
  - Abortion Necropsy, reviewer
  - Adult diarrhea/Weight Loss Necropsy, reviewer
  - BRD Necropsy, reviewer
  - Calf Diarrhea Necropsy, reviewer
  - CNS Necropsy, reviewer
  - General Necropsy, creator
  - Introduction to Section, creator
  - Necropsy Field Kit Assembly, reviewer
  - Sudden Death Necropsy, reviewer
4. **Terry Hensley, MS, DVM**  
Assistant Agency Director, Veterinary Services
  - Abortion Necropsy, reviewer
  - Adult diarrhea/Weight loss Necropsy, reviewer
  - BRD Necropsy, reviewer
  - Calf Diarrhea Necropsy, reviewer
  - CNS Necropsy, reviewer
  - General Necropsy, reviewer
  - Sudden Death Necropsy, reviewer
5. **Jay Hoffman, DVM, PhD, DACVP**  
Section Head, Pathology, TVMDL-College Station, Retired
  - General Necropsy, reviewer
6. **Jessie Monday, DVM, MS**  
Veterinary Diagnostician, TVMDL-Canyon
  - Section III Coordinator & Editor
  - Abortion Necropsy, creator
  - Adult diarrhea/Weight loss Necropsy, creator
  - BRD Necropsy, creator
  - Calf Diarrhea Necropsy, creator
  - CNS Necropsy, creator
  - General Necropsy, creator
  - Introduction to Section, editor
  - Necropsy Field Kit Assembly, editor
  - Necropsy Supply Glossary, creator
7. **Mallory Pfeifer**  
Communications Specialist
  - Media Dept Support
  - Sample Packaging for Shipment, creator
  - Shipping Box Packing Guide, editor
8. **Sandy Rodgers, MS**  
Section Head, Serology, TVMDL, Retired
  - Abortion Necropsy, reviewer
  - Adult diarrhea/Weight loss Necropsy, reviewer
  - BRD Necropsy, reviewer
  - Calf Diarrhea Necropsy, reviewer
  - CNS Necropsy, reviewer
  - Sudden Death Necropsy, reviewer
9. **Guy Sheppard, DVM**  
Veterinary Diagnostician, TVMDL-College Station
  - Abortion Necropsy, creator
  - Adult diarrhea/Weight loss Necropsy, creator
  - BRD Necropsy, creator
  - Calf Diarrhea Necropsy, creator
  - CNS Necropsy, creator
  - General Necropsy, reviewer
  - Necropsy Field Kit Assembly, creator
  - Sample Packaging for Shipment, reviewer
  - Shipping Box Packing Guide, reviewer
  - Sudden Death Necropsy, creator
10. **Will Sims, DVM, MS**  
Pathologist, TVMDL-Canyon
  - General Necropsy, reviewer
  - Necropsy Field Kit Assembly, reviewer