



BOVINE DISEASE DIAGNOSTIC GUIDE

A NEW ERA OF VACCINE TECHNOLOGY



DIAGNOSTIC SPECIMENS AND TESTS

| Disease Suspected | Specimen | Sample Preparation | Laboratory Procedure |
|--|---|--------------------|---|
| Actinomycosis | Abscessed Tissue | 10% Formalin | Histopathology & Tissue Stains |
| | Abscessed Tissue & Exudate | Refrigerate | Anaerobic Culture |
| | Anaerobic Culture Swab | Refrigerate | Anaerobic Culture |
| Bovine Herpes Virus 1 (IBRV) | Lung, Trachea, Nasal Swabs | Refrigerate | PCR, Virus Isolation |
| | | 10% Formalin | Histopathology |
| Bovine Respiratory Syncytial Virus (BRSV) | Lung | Refrigerate | PCR, Virus Isolation |
| | | 10% Formalin | Histopathology |
| Bovine Virus Diarrhea (BVDV) | Lung, Ileum, Cecum, Spiral Colon, Mesenteric Lymph Nodes, Spleen, Whole Blood (Purple top tube) | Refrigerate | PCR, Virus Isolation, Gene Sequencing |
| | | 10% Formalin | Histopathology |
| Calf Diphtheria | Lesion Material, Swab from Lesion | Refrigerate | Anaerobic Culture |
| Caseous Lymphadenitis (sheep & goats) | Purulent Exudate from Abscess | Refrigerate | Culture, Sensitivity |
| Clostridium difficile | Colon (sections with contents) | Refrigerate | Anaerobic Culture, A/B Toxin ELISA |
| Clostridium perfringens Types A, C, D, E | Small Intestine (tied off), Abomasum (fresh), Liver (fresh) <i>Note: Do not submit fecal samples</i> | Refrigerate | Anaerobic Culture, Sensitivity, Toxin PCR Testing |
| | | 10% Formalin | Histopathology |
| Coccidiosis/ Cryptosporidiosis | Small Intestine (with Cecal & Spiral Colon & Contents), Feces | Refrigerate | Fecal Float, Stain |
| | | 10% Formalin | Histopathology |
| Colibacillosis | Small Intestine (Jejunum, Ileum, & Colon), Feces from calves less than 7 days old. | Refrigerate | Culture & K99 Pilus Identification |
| | | 10% Formalin | Histopathology |
| Colisepticemia | Lung, Liver, Spleen, Kidney, & Lesions | Fresh | Culture, Sensitivity |
| | | 10% Formalin | Histopathology |
| Coronavirus (respiratory) | Lung, Nasal Swabs | Refrigerate | PCR, Virus Isolation |
| Enteritis (Rotavirus and Coronavirus) | Intestine (with Cecal & Spiral Colon Section & Contents) | Refrigerate | PCR, Virus Isolation, Sequencing |
| | Feces | | PCR, Virus Isolation, Sequencing |
| Footrot (Fusobacterium necrophorum) | Swab from Lesion | Refrigerate | Anaerobic Culture, PCR Subspeciation |
| Hardware Disease | Reticulum, Pericardium | 10% Formalin | Histopathology |

For bacterial culture we recommend swabs with transport media to prevent desiccation.
For Virus Isolation, swabs should be placed into viral transport media; call us for more information.

Samples intended for Custom Made Vaccine production should be refrigerated. These samples should not be fixed in formalin.

MAJOR BOVINE DISEASES

| Disease Suspected | Specimen | Sample Preparation | Laboratory Procedure |
|--|--|-----------------------|--|
| <i>Histophilus somni</i> (formerly <i>Haemophilus somnus</i>) | Affected Lung | Refrigerate | Culture, Sensitivity, Gene Sequencing, PCR for Immunoglobulin Binding Proteins |
| | | 10% Formalin | Histopathology |
| | Myocardium, Brain | Refrigerate | Culture, Sensitivity, Gene Sequencing, PCR |
| | | 10% Formalin | Histopathology |
| | Joint Swabs (if involved) | Refrigerate | Culture, Sensitivity, Gene Sequencing, PCR |
| Interstitial Pneumonia | Lung, Ventral & Dorsal Areas | Fresh | Bacteriology & Virology |
| | | 10% Formalin | Histopathology |
| Johne's Disease | Ileum | 10% Formalin | Histopathology, Acid-Fast Stain |
| | Ileum or Feces | Fresh, Refrigerate | Johne's Culture, PCR at Reference Lab |
| <i>Mannheimia haemolytica</i> (formerly <i>Pasteurella haemolytica</i>) | Affected Lung, Lymph Nodes (mediastinal or bronchial) | Refrigerate | Culture, Sensitivity, Gene Sequencing, Serotyping |
| | | 10% Formalin | Histopathology |
| Mastitis | Milk Sample from Affected Quarter or Bulk Tank | Refrigerate or Frozen | Culture, Sensitivity |
| <i>Mycoplasma bovis</i> | Affected Lung | Refrigerate | Mycoplasma Culture, Speciation, Gene Sequencing |
| | | 10% Formalin | Histopathology |
| | Joint Swabs/Fluids, Tracheal Swabs, Swabs or Exudate from Middle Ear | Refrigerate | Mycoplasma Culture, Speciation, Gene Sequencing |
| Parainfluenza-3 Virus (PI-3) | Lung, Nasal Swabs | Refrigerate | PCR, Virus Isolation |
| | | 10% Formalin | Histopathology |
| <i>Pasteurella multocida</i> | Affected Lung, Lymph Nodes (mediastinal or bronchial) | Refrigerate | Culture, Sensitivity, Typing |
| | | 10% Formalin | Histopathology |
| Pinkeye (<i>Moraxella bovis</i>, <i>M. bovoculi</i> & <i>M. ovis</i>) | Eye Swabs | Refrigerate | Culture, Sensitivity, Gene Sequencing |
| Polioencephalomalacia | Cerebral Cortex, Brainstem | 10% Formalin | Histopathology |
| | Water, Feed/Forage | Fresh | Sulfate Analysis at Reference Lab |
| Pyelonephritis | Kidney, Urinary Bladder | 10% Formalin | Histopathology |
| | Kidney, Urinary Bladder, Urine (fresh) | Refrigerate | Bacterial Culture |
| Reproductive failure/abortion | Fetus with Placenta (if available) | Refrigerate | Reference Lab |
| Salmonellosis | Small Intestine, Spleen, Lung, Liver, Mesenteric Lymph Nodes: (all fresh and in formalin;) Fresh Feces & Fecal Swabs | Refrigerate | Culture, Sensitivity, Gene Sequencing |
| | | 10% Formalin | Histopathology |
| Trace Mineral Deficiency (i.e. copper & selenium) | Liver (fresh) | Refrigerate | Reference Lab |

For bacterial culture we recommend swabs with transport media to prevent desiccation.
For Virus Isolation, swabs should be placed into viral transport media; call us for information.

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SELECTION OF SPECIMENS

Whenever possible, animals selected for laboratory analysis should be free from antibiotic therapy and in an early or acute disease stage. Selected tissues should be collected as aseptically as possible. A meaningful history of the disease outbreak and a tentative diagnosis, based upon clinical evaluation and necropsy findings, should be included. Laboratory test results are directly affected by animal selection, necropsy technique, specimen selection and specimen handling, including preservation and shipment to the laboratory. Contact Newport Laboratories, Inc. if you have any questions regarding sample collection or the diagnostic process.

Preparation and Collection of Tissues/Samples

Tissues-Fresh

Aseptically collect approximately 2x4 inch samples and place in a plastic bag. Sample visible lesions with adjacent normal tissue. Double bag in Whirl-pak® bags. Do not mix swabs, intestines, or brains with other tissues in one single bag. Transport tissues with 2-3 cold packs in an insulated container. It is important that the tissue samples arrive at the laboratory before the cold packs expire.

Collect sections of small and large intestine. The selected, clearly identified samples should be double bagged and sealed in Whirl-pak bags to prevent spillage. Do not longitudinally cut the loops of intestines open. The intestine, approximately 2 inches long, should be refrigerated and cooled thoroughly prior to shipping. Avoid shipping over the weekends or holidays.

Swabs

Aerobic Culture

Commercial swabs with Stuart's or Amies transport media is recommended to prevent desiccation.

Anaerobic Culture

Port-A Cult® (BBL) or other anaerobic transport system. (The Port-A Cult® tube can be used for anaerobic, facultative, and aerobic bacteria.) For abscesses or exudates use a capped syringe with needle removed or a tube with a snug cap. Cary Blair Culturettes are recommended for *Fusobacterium necrophorum*.

Nasal Swabs-Bacterial Suspect

Clean the external nares and internal nostrils with a moist towel to remove common contaminants. (Use swabs with transport media such as Amies or Stuart's). Insert swab into the pre-cleaned nasal cavity and rotate. Upon successful sample collection, the swab is inserted into the accompanying sterile plastic sheath. The ampule located at the end of the sheath is gently crushed, releasing transport medium.

Nasal Swabs-Viral Suspect

Prepare nostrils and sample as in bacterial suspect. For viral swabs use Viral Culturette® (Becton Dickinson #220221) or equivalent.

Use of the incorrect swab and media may jeopardize the ability to detect or culture the offending pathogen. For bacterial isolation, avoid using Mycoplasma sp. or viral media which contain antimicrobials and may inhibit growth of the desired pathogen. Avoid using bacterial culture media to isolate viruses or Mycoplasma sp. organisms.

Identify all swabs with the following:

- Farm ID, including site and pasture/lot where appropriate
- Animal identification number

Histopathology

Preparation of Tissue for Fixation

Multiple sites or types of lesions, to include both normal and diseased tissue and a sample at the line of demarcation, should be taken. **The sections should be no more than 1 inch thick. The small size of the tissue results in rapid and complete penetration of the fixative.**

Selected tissues should be cut with a sharp knife or scalpel since the squeezing action of scissors crushes and tears tissue. Autolysis or freezing will make samples unsuitable for histopathological evaluation. Place formalin and tissues in double Whirl-paks. Identify bags if multiple animals are submitted. Do not use narrow mouth bottles to submit fixed tissues.

Volume of Fixative

The selected tissues should be fixed in 10% neutral buffered formalin. Use 10 times the volume of the tissues being fixed to assure good perfusion of the sample and to maintain the tissue architecture. After 24 hours fixation, excess formalin can be poured off, and a smaller formalin volume can then be used for shipping.

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Formula to make 10% Neutral Buffered Formalin

| | |
|--|--------|
| 37-40% formaldehyde | 100 mL |
| Distilled water | 900 mL |
| Sodium phosphate, monobasic monohydrate (NaH ₂ PO ₄ ·H ₂ O) | 4.0 g |
| Sodium phosphate, dibasic anhydrous (Na ₂ HPO ₄) | 6.5 g |

Tissue Selection for Histopathology

Check the recommended samples in the guideline table of this detailer. If the cause of death is unknown or the clinical syndrome is vague, then submit samples exhibiting suspected gross lesions and the following tissues: heart, liver, lung, kidney, spleen, various levels of the gastrointestinal tract, mesenteric lymph nodes, and brain.

If hollow organs (gut or uterus) retain significant amounts of content, then they should be gently flushed with 10% formalin without disturbing the mucosal lining before placing in the formalin bag. Be sure to take proper precautions when handling formalin.

I.D. & Handling of Blood Samples:

Collection of Blood Samples

- Collect in sterile tubes. Serum separator tubes work well. Follow the manufacturer's directions. Based on the number of tests requested, 1 mL – 3 mL of nonhemolyzed serum is required.
- Fill vacutainer tubes $\frac{3}{4}$ full and allow to stand at room temperature for an hour to permit a solid clot to form and retract.
- Pipette the serum into sterile tubes with snap caps (5 mL plastic tubes with snap caps, Falcon® #352054, are recommended). Make sure caps are securely closed.
- Use permanent markers and underline the I.D. numbers (e.g. 16 vs. 91).
- Do not freeze whole blood or samples with the clot remaining.
- Contaminated or toxic samples cannot be used in virus isolation tests. Many serology tests are adversely affected by hemolysis.

I.D. Samples on Submission Forms

- Using one form per client and site, identify the tubes on the submission request form by different barns, or age groups as logical for the diagnostic investigation.
- Clearly specify the test(s) requested on the submission form.
- When sending paired sera, identify the acute samples from the convalescent samples on the tube and on the request form.

Diagnostic submission forms can be downloaded from our website: www.newportlabs.com, or by calling Newport Laboratories, Inc. customer service at 800-220-2522.

Packing Specimens

To avoid leaking in transit, double bag ALL samples. Whirl-pak bags or equivalent are recommended. Wrap sample bags and 2-4 ice packs in absorbent paper (e.g. newspaper). Place the package into a styrofoam container. Completed submission forms should be inserted in a separate bag in case of leakage and clearly attached to the matching specimens. This is especially important if your container contains specimens from multiple clients or sites. Avoid mixing intestinal samples with other tissues. If you need more information about shipping specimens to Newport's Diagnostic Laboratory, please call us at 800-220-2522.

Mailing

Newport Laboratories, Inc. provides free diagnostic kits for sample submission. Call us at 800-220-2522 to request submission form(s) or shipping containers. Submission forms are also available online at www.newportlabs.com. Samples should be submitted by the fastest means possible to avoid deterioration of specimens. Next day or overnight delivery is preferred. The most reliable services that we have found are listed below:

- FedEx®
- United Parcel Service (UPS)
- Spee-Dee
- U.S. Parcel Post (only as a final option)

Flat Rate Shipping

Take advantage of our flat rate shipping program and save on your shipping costs. Order your diagnostic shippers today either online at www.newportlabs.com or by calling 800-220-2522.

Laboratory Hours

The Newport Diagnostic Laboratory is open for service from 8:00 a.m. to 5:00 p.m. (CST) Monday through Friday, with the exception of holidays.

Diagnostic Shipping Address



1520 Prairie Drive
Worthington, MN 56187

SPECIFIC DISEASE SAMPLING SUGGESTIONS AND TESTS

Mycoplasma bovis

Recommended tissues/samples for culture:

- Affected lung (often, but not always, abscessed)
- Bronchial alveolar lavage samples or deep tracheal swabs are optional methods to obtain respiratory isolates antemortem
- Joint swabs from enlarged joints
- Swabs or exudate from middle ears (of drooped ears)
- Nasopharyngeal swabs or nasal swabs (from sick animals exhibiting clinical signs)

(Note: nasal swabs should be used only in the absence of other tissues/samples. Although *Mycoplasma spp.* may be present in the nasal passages, isolates obtained by this method may not necessarily be representative of the strains causing disease in the lower respiratory tract.)

Recommended tissues/samples for histopathology (if desired): affected lung

Pinkeye (Moraxella bovis, M. bovoculi and M. ovis)

- Select acutely infected eyes that have not been treated systemically or topically. Acute infections (in the early excessive lacrimation stage, prior to development of corneal lesions) are more likely to yield positive cultures.
- Using a Culturette® swab (Amies transport media works well) or other sterile swab, sweep aggressively under the lower conjunctiva, using both a rolling and a swabbing motion to obtain a liberal amount of lacrimal fluid.
- Once the sample has been obtained, the swab may be either inserted into the Culturette tube (Amies media) or swabbed directly onto a blood agar plate. The Culturette or plate should be submitted, on ice, to Newport Laboratories, Inc. as soon as possible.
- Multiple samples are recommended to increase the chances of isolating the organism.

(Note: if Mycoplasma or viral involvement, typically BHV, is suspected, the affected eye(s) should also be swabbed with a viral swab which is then inserted into a viral transport media. Immediate refrigeration or freezing of the swabs is recommended.)

Reproductive Failure/Abortion

- Submit fresh placenta, with special attention to affected areas with abnormal appearance.
- Deep vaginal swabs, or swabs of cervix or uterus using a guarded pipette/swab, e.g. Tieglund swab. Use both Amies or Stuart's medium for transport, as well as viral swabs and transport media for possible ureaplasma detection.

Mastitis

- Submit milk samples from affected quarters or the bulk tank (a few mL is adequate) and submit on ice. Frozen samples are fine. Samples can be cultured for bacteria, Mycoplasma and Prototheca.

Reclassification of Bacterial Isolates or Identified New Strains¹

| Old Nomenclature | New Nomenclature or Strain |
|---------------------------------|-------------------------------|
| Respiratory | |
| <i>Pasteurella haemolytica</i> | <i>Mannheimia haemolytica</i> |
| <i>Haemophilus somnus</i> | <i>Histophilus somni</i> |
| <i>Pasteurella trehalosi</i> | <i>Bibersteinia trehalosi</i> |
| | |
| Pinkeye | |
| <i>Moraxella ovis</i> | <i>Moraxella bovoculi</i> |
| | |
| Mastitis | |
| <i>Arcanobacterium pyogenes</i> | <i>Trueperella pyogenes</i> |

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References:

1. Holt J.G. Bergey's Manual of Determinative Bacteriology. 9th ed. Baltimore, MD: Williams & Wilkins; 1994.

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