NORTH CAROLINA VETERINARY DIAGNOSTIC LABORATORY SYSTEM

User Guide
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North Carolina Department of Agriculture and Consumer Services

Steven W Troxler, Commissioner of Agriculture
Michael P Martin, DVM State Veterinarian
James Trybus, DVM, DACVP, Director of Laboratories

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NCVDLS Website Link:
ncagr.gov/vet/ncvdl/

I. Introduction

The North Carolina Veterinary Diagnostic Laboratory System (NCVDLS) was established in 1947 by the North Carolina Department of Agriculture. Our mission is to provide veterinarians, the animal industries, and the citizens of North Carolina with accurate and timely laboratory support services to diagnose, conduct surveillance, and assist in responding to and preventing animal disease. Protection of both public health and the food supply are important components of this mission. The laboratory is fully accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD) and offers services in bacteriology, molecular diagnostics, mycology, parasitology, pathology, serology, and virology and limited services in toxicology. Most Chemistry/Toxicology services are outsourced to the Pennsylvania Animal Diagnostic Laboratory System.

Rollins Laboratory in Raleigh is the full-service central laboratory, with branch laboratory locations in Fletcher, Elkin, and Monroe. The laboratories are open Monday through Friday, 8 a.m. to 5 p.m. Emergency after hours, weekend, and holiday submissions must be arranged by contacting the individual laboratory.

Contacting the Laboratory

Many answers to your questions may be found in this guide. Please feel free to call with any additional questions. When calling for preliminary results, please be prepared to provide us with the following information so we can assist you more quickly:

- Case accession number (if known)
- Name/address/phone number of submitter
- Owner's name
- Animal identification
- Date of submission

NCVDLS Facilities

Rollins Animal Disease Diagnostic Laboratory

Director of Laboratories – Dr. James Trybus
Assistant Director of Laboratories – Dr. Lalitha Peddireddi
Pathology Services Coordinator – Dr. James Trybus
Mammalian Pathologists – Dr. James Trybus, Dr. Steve Rushton, Dr. Ida Phillips
Avian Pathologist – Dr. Tahseen Aziz
Histopathology Section Head – Dr. James Trybus
Bacteriology Section Head – Dr. Anil Thachil
Toxicology Section Head – Dr. Cat Barr
Virology/Serology Section Head – VACANT
Molecular Diagnostics Section Head – Dr. Lalitha Peddireddi
Veterinary Diagnosticians – Dr. Mahogany Caesar, Dr. Jennifer Haugland, Dr. Stacy Robinson

FedEx/UPS: 4400 Reedy Creek Road, Raleigh, NC 27607
US Mail: 1301 Mail Services Center, Raleigh, NC 27699-1031
Phone: (919) 733-3986 Fax: (919) 733-0454

Western Animal Disease Diagnostic Laboratory
Resident Director – VACANT
Veterinary Diagnostician – Dr. Mary Swanson
785 Airport Road, Fletcher, NC 28732
Phone: (828) 684-8188 Fax: (828) 687-3574

Northwestern Animal Disease Diagnostic Laboratory
Resident Director – Dr. Kimberly K Hagans
Veterinary Diagnostician – VACANT
1689 N. Bridge Street, Elkin, NC 28621
Phone: (336) 526-2499 Fax: (336) 526-2603

Hoyle C. Griffin Animal Disease Diagnostic Laboratory
Resident Director – Dr. Heather Wyss
Veterinary Diagnostician – Dr. Kristine Blankenship
401 Quarry Road, Monroe, NC 28112
Phone: (704) 289-6448 Fax: (704) 283-9660

Referrals

The procedure for clients requesting tests that are not performed at an NCVDLS facility is to inform them of the situation. At the request of the client, samples will be shipped to another laboratory and tracked in our system. The client account will be billed $20 for shipping and handling and the client is responsible for fees associated with testing whether they are billed by us or by the outsourced laboratory.
Note: Caseous lymphadenitis (CL) testing is not available at Rollins lab. When serum samples are submitted for multiple tests including CL, client should indicate in the submission form if they would like to outsource CL testing.

National Animal Health Laboratory Network and Other 'Official' Testing

The Rollins facility is a Tier 1 laboratory and core member of the National Animal Health Laboratory Network (NAHLN) which is a cooperative effort between two USDA agencies, the Animal and Plant Health Inspection Service (APHIS) and the National Institute of Food and Agriculture (NIFA), and the American Association of Veterinary Laboratory Diagnosticians (AAVLD).
Participating laboratories perform routine diagnostic tests for endemic animal diseases, as well as targeted surveillance and response testing for foreign animal diseases. Consequently, samples may be tested for surveillance purposes (see fee schedule).
Official regulatory tests may be required by state, federal and/or international agencies for the movement or certification of animals. NCVDLS facilities are approved by the National Veterinary

Services Laboratory to perform a variety of official tests. For these types of tests, please ensure that your submission meets any official requirements including being accompanied by an official test chart signed by an accredited veterinarian. Please refer to our Test Schedule (part V) to determine the need for this.
Current import/export requirements are available by contacting the USDA office (919) 855-7700.

II. Submitting Specimens, Submission Forms, Shipping Regulations, and Sample Retention

Submitting Specimens

Specimens can be delivered in person, by commercial courier service, such as, FedEx, UPS or U.S. Post Office. **We strongly discourage the shipment of perishable specimens by US Post Office because this delivery method is slower and may compromise specimen quality.** NCVDLS has contracted discounted rates with both FedEx and UPS for packages weighing 5 lbs. or less. A flat fee of $10.00 per shipment can assure overnight delivery. The fee is applied to client accounts each time the service is used. Contact the Rollins Laboratory Business Office at 919.733.3986 for more information.

Shipping Regulations

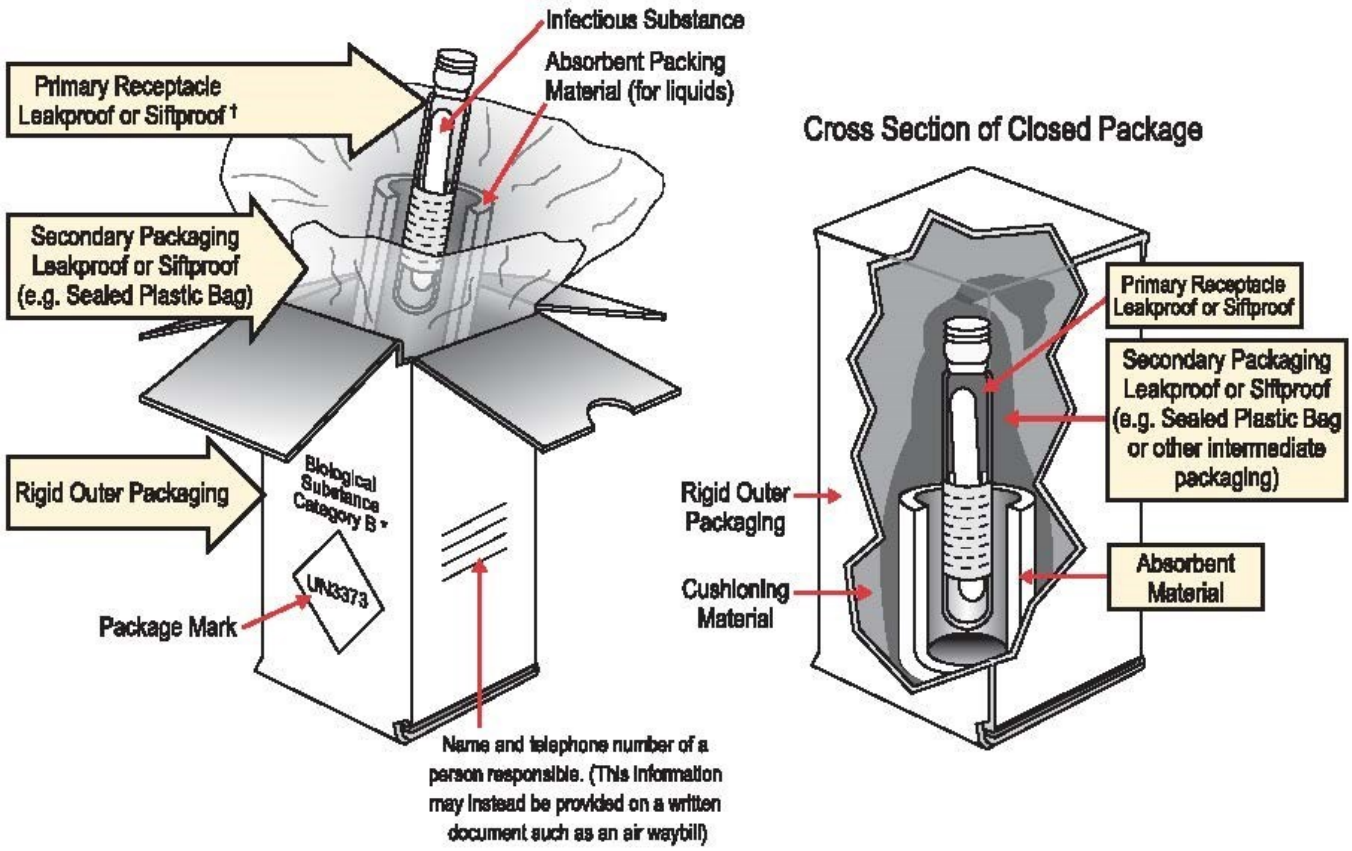
Laws in effect since February 2003 have affected the definition of diagnostic specimens and how they are classified, packaged, and transported. These rules apply to the shipment of veterinary specimens to diagnostic laboratories via commercial carriers such as FedEx or UPS. The rules are mandated at the Federal level rather than the state or NCVDLS level. Veterinarians are subject to these rules, and non-compliance can result in very stiff fines. More stringent requirements are in effect for known infectious agents.

Formalin-fixed tissues are exempt but should still be packaged in leak-proof containers with adequate absorbent material.

It is vital that clients who package diagnostic samples for shipment to the laboratory have the required documented training and ensure that each shipment meets current packaging standards that are mandated by the IATA and USDOT. Please refer to these websites for additional information.

- www.phmsa.dot.gov/staticfiles/PHMSA/DownloadableFiles/Files/Transporting_Infectious_Substances_brochure.pdf
- www.iata.org/whatwedo/cargo/dangerous_goods/Pages/infectious_substances.aspx
- www.avma.org/issues/pack_ship_lab_specimens.asp

Potentially hazardous biological materials must be packaged to withstand content leakage, shocks, temperature changes, pressure changes, and other conditions that can occur during transport. All biological materials must be tripled packaged as diagrammed below.



* *The proper shipping names "Biological Substance, Category B"; "Clinical Specimen"; and "Diagnostic Specimen" are authorized until December 31, 2006. From January 1, 2007 only the proper shipping name "Biological Substance, Category B" will be authorized.*

† *If multiple fragile primary receptacles are placed in a single secondary packaging they must be either individually wrapped or separated to prevent contact*

Note: *Follow package manufacturer's closure instructions*

Please choose a carrier that will deliver specimens within 24-48 hours of sample collection. Please avoid weekend and holiday deliveries, if at all possible. Please contact the Rollins Laboratory business office for more information (919) 733-3986.

Submission Forms

A completed submission form must accompany specimens submitted to NCVDLS facilities. Forms may be downloaded from the NCVDLS website: https://www.ncagr.gov/vet/ncvdl/VetLabSubmissionForms.html.

- Avian/Poultry Submission Form**:** Use for all avian specimens including tissues, live/dead birds, biopsy, and cytology samples.
    - Exception: Serum specimens submitted for avian influenza*, Mycoplasma gallisepticum, M. synoviae, M. meleagridis* testing in association with the National Poultry Improvement Plan must also be accompanied by the appropriate NCDA/Vet Division form F041.

- Surgical Biopsy and Cytology Submission Form: Use for all cytology specimens (fluid or slides) and biopsy samples obtained from live animals. Bacterial or fungal culture of these samples can also be ordered using this form.
- General Submission Form: Use for all other types of specimens, including serum, tissues, swabs, necropsy specimens.
- Swine Test Chart: Use for PRV testing. Contact Rollins to request forms.

Fill out the forms completely including owner information and animal identification. Please list specimens being submitted and list test requests in the appropriate blanks. The User Guide will help to identify test offerings. **If submission forms are incomplete or if specific tests are not requested, specimen processing/testing will be delayed until the necessary information is provided.**
At the time of receipt, specimens are assigned an accession number. This number is used to track the specimen throughout the laboratory. As a general rule, a case coordinator is assigned to the accession at this time and he/she is responsible for the specimen and for reporting test results.

Sample Retention

Specimens submitted to the laboratory become the property of the NCVDLS and may be subject to additional diagnostic testing for state and federal disease surveillance programs.
Sample retention policies are listed below by testing section. Please notify the laboratory *at the time of submission* if longer retention is required.

Necropsy	Carcasses are disposed of following examination unless private cremation is requested. Based on diagnostic necessity and carcass conditions, tissue samples may not be retained. When retained, fresh tissue samples may be stored for 1 month from the date the animal is received. Formalin fixed and frozen tissue samples may be stored for up to 2 months.
Histopathology, Biopsies, and Cytology	Formalin-fixed tissue is retained for 2 weeks. Paraffin embedded tissue blocks are retained for 7 years. Cytology samples are retained for 1 month.
Bacteriology/Parasitology	Samples are generally retained for 2 weeks from the date received unless there is insufficient sample or if sample is forwarded to another department. Milk samples may be frozen and retained for 1 month.
Serology	Chicken and turkey samples for Mg/Ms/Mm are saved for 2 weeks. All Mammalian ELISA samples are saved for 2 weeks. Samples for Brucellosis testing are saved for 1 week unless forwarded to another department. Samples for Leptospirosis testing and saved for 4 weeks.
Virology	Samples are retained for 1 month if space allows.
Molecular Diagnostics	Samples are retained for 1 month if space allows.
Toxicology	Samples are generally retained for 2 weeks unless forwarded to another laboratory for testing.

III. Test Results, Fees, and Billing

Test Results

NCVDLS reports of test results can be distributed by

- Fax
- US Mail
- Web Portal – Clients will need an account with username and password to log in to the web portal. An email will be sent to the client to notify them the report is ready to be viewed and downloaded from the website https://lims.ncvdl.com/NCVDLSOnline/Login.aspx. Reports are ready once results are distributed by the case coordinator/section head.
- Email – Reports that are sent by email are attached as a PDF document and thus are not secure. Since the laboratory has a responsibility to protect client confidentiality, we require a signed waiver of acknowledgment that this method is not secure.

Please contact the Rollins Laboratory business office to request a waiver and to set up email as the method of report distribution or with other questions about report distribution.

NCVDLS recognizes the importance of test results to our clients. If you have questions about a test result, please call us, referencing the laboratory accession number. Turn-around times for our test services appear in the NCVDLS test schedule section of this User Guide. Estimated turn-around times are counted in business days from the date of specimen receipt until the date that results are reported, assuming there are no complications with testing.

Fees

A current Fee Schedule is available on our website at ncagr.gov/vet/ncvdl/ and fees are subject to change without notice.

Billing

Clients are assigned an account number. This account number should be referenced for all billing inquiries to the Rollins Laboratory Business Office. Clients are responsible for payment of all billable services. Please check the Bill To box for the appropriate client on the submission form. If a third party is to be billed, please indicate that on the submission form. Invoices are generated at the end of each month and transmitted to the client for payment. The State of North Carolina requires that all debts be paid within 90 days. Late fees are mandated on accounts that are 30 days past-due at an interest rate of 5%. Accounts with balances over 90 days past-due are placed on hold and no services can be rendered until bills are paid.

IV. Rollins Laboratory Sections

A. Pathology

Pathology services include performing diagnostic necropsies, histopathological evaluations of tissue samples, immunohistochemistry, and cytology services. All laboratories within the

NCVDLS offer diagnostic necropsies. Histopathology, immunohistochemistry, and cytology services are performed exclusively at the Rollins Laboratory. Telephone consultation with a pathologist or diagnostician prior to submissions is particularly encouraged if a case is complex or if the diagnostic investigation involves an ongoing series of submissions.

Necropsy Services

Necropsies are performed at each laboratory from 8 a.m. to 5 p.m., Monday through Friday. Animals received late in the day may not be examined until the following day.

Animals should be submitted as soon as possible after death. Live animals are preferred only in the following situations: porcine neonatal diarrhea, and diseases of poultry and fish. The laboratories do not euthanize companion animals or horses. Euthanasia of cattle, sheep, goats and camelids is only done when the animal is unable to stand and does not struggle. Transporting down cattle is often not humane and so euthanasia prior to transportation is strongly encouraged. There is an additional fee for euthanasia at the laboratories. A frozen body does not prevent a necropsy however it may cause additional decomposition, freezing artifact and may prevent the isolation of pathological agents. Refusal of an animal for necropsy at any time is at the discretion of the assigned veterinarian and will be based upon the following: (a) an animal is deemed too decomposed for further diagnostic testing, (b) an animal has clinical signs that are consistent with a recent laboratory diagnosis, therefore an additional necropsy is unwarranted, (c) a diagnosis has already been obtained and confirmed, therefore a necropsy is unwarranted (e.g. fractured leg, uterine prolapse, chronic laminitis). **Animals refused for necropsy will not be accepted by the laboratory for disposal only.**

Use the General Submission Form to submit animals for necropsy. Complete the Veterinarian contact information, owner contact information, the animal identification section and write a thorough history. The history is most helpful when it is written by the attending veterinarian.

After Hours

Necropsies are generally not performed on State holidays, unless they qualify as an emergency. At the Rollins Laboratory facility only, routine weekend necropsies are limited from 8 a.m. to 12 p.m. on Saturday. No routine necropsy services are available on Saturday at the branch laboratory facilities. Emergency situations are limited to (a) cases of multiple deaths within a flock/herd over a short period of time (24/48 hours), (b) cases of suspected foreign animal diseases (Foot and Mouth, Exotic Newcastle, etc.), (c) zoonotic diseases.

An 'on-call' veterinarian is available at Rollins lab facility after hours by telephone for consultation purposes. An after-hours cooler is available outside the Rollins Laboratory for animals less than approximately 150 lbs. Clients with non-emergency cases will be encouraged to have animals necropsied by their own veterinarian, as decomposition may preclude the ability to make a diagnosis.

Animal Remains

Animal remains **remains are prohibited to be returned to the owner or veterinarian by**

NC statute (02NCAC52C.0302)**.** An exemption has been made for small companion animals to be cremated by commercial crematory services for ashes to be returned to owner. Water cremation services are also permitted. Saving the body for private cremation **must be requested by notation on the submission form at the time of submission**. Clients are responsible for making their own crematory arrangements. The fee for disposal is waived but the client is responsible for the charges from the crematory service. Cremation of small livestock animals (pot-bellied pigs, goats, sheep, camelids) and a horse's head and heart may be possible depending on the crematory service and size of the animal. If an animal is tested for rabies virus or a horse is tested for encephalitis, the body/head and heart will not be released to a crematory until the tests are found to be negative. **If any animal is positive for rabies or encephalitis in a horse, the entire body will be mass cremated at Rollins Laboratory and will not be released for private cremation due to safety concerns of the crematory personnel.**

Results
Preliminary gross findings of the necropsy examination are generally reported to the client within 24-36 hours of completion. Often, additional laboratory procedures are required to arrive at a diagnosis. A final written report will be distributed to the client upon conclusion of the investigation.

Legal/Cruelty Cases
Animal cruelty/legal cases will be handled in the same manner as a routine necropsy submission. Additional forensic testing such as determining the time of death, forensic entomology, or determining types of accelerants used in burning cases is not performed. Please notify the laboratory at the time of submission if there is a potential cruelty or a legal issue. Any pictures of the animal must be taken prior to laboratory submission. When poisoning is suspected, please notify the laboratory at the time of submission. Samples may be outsourced for toxicology testing at the client's request with the client being responsible for associated costs. If bullet retrieval is desired in suspected gunshot cases, radiographs should be taken and brought to the laboratory at the time of submission.

Rabies Testing
Testing is performed on fresh brain tissue by the North Carolina State Laboratory of Public Health located in Raleigh. The NCVDLS is not to be utilized for the transshipping, case management, or client notification of companion animal and wildlife/feral rabies suspects involving human exposure. **If only rabies testing is desired**, veterinarians or owners should contact their county Animal Control or Health Department. More information about rabies can be found at https://epi.dph.ncdhhs.gov/cd/lhds/manuals/rabies/testing.html or by calling (919) 733-7544 during regular hours of operation (7:30am-4:30pm weekdays).

Field Necropsy Guidelines
When collecting tissues in the field, the history, clinical signs, and gross lesions should determine which tissues to collect. Proper selection and preservation of samples is critical. **Please call the appropriate laboratory and speak to a veterinary pathologist or diagnostician at any time if you have questions concerning specimen selection, collection, or transport.**

1) Specimens for histopathology should include multiple slices of appropriate organs, including lesions, transitional zones, and adjacent grossly normal tissue. When in doubt, collect specimens from multiple organs, including brain. For feline cardiomyopathy cases, submit the entire opened heart. Specimens should be less than ¼" thick (formalin penetrates only ⅛ " in the critical first 24 hours of fixation) and placed in leak-proof, wide-mouthed solid containers with a formalin-to-tissue ratio of 10 to 1. **NOTE**: specimens should NOT be refrigerated during the fixation process, as this will delay penetration of formalin into the tissues.
2) Fresh specimens should be large enough to demonstrate the lesion yet small enough to allow for rapid chilling. Fresh samples should be packaged individually to prevent cross-contamination in leak proof containers and properly labeled. Tied off loops of intestine or containers of intestinal contents are preferred over swabs. Tied off loops of intestine must be packaged separately from other tissues.
3) See test schedule for additional information about preferred samples and preservation.

The three conditions that most frequently interfere with a diagnosis are
1) Advanced postmortem autolysis
2) Sample collection too late in the course of disease
3) Inappropriate sample selection.

With each submission, include signalment, history, differential diagnoses, and provide a list of specimens.

Biopsy and Cytology Services

Submission of surgical biopsies, aspirated fluids and slides made from aspirates should be accompanied by a completed Surgical Biopsy and Cytology submission form. Including the following information is crucial for in-depth and accurate interpretation of the samples: **1)** animal name and signalment (age, breed, and sex); **2)** the method used to obtain the sample (e.g. excisional biopsy, incisional biopsy); **3)** information pertaining to the location, size, duration of the lesion and response to treatment, if any. For smaller tissue samples, fixation is complete within 24 hours and a smaller volume of formalin may be used for shipping. Surgical biopsies should be submitted in one piece for margin evaluation. Larger samples can be partially incised through the skin (not the surgical margins) to enhance fixation.

The preferred sample for cytologic evaluation is a smear of the aspirate on glass microscope slides. Samples such as trans-tracheal washes and effusions can be submitted in an EDTA tube (EDTA samples are not suitable for bacterial culture). DO NOT submit cytology slides in the same package with samples in formalin because formalin fumes cause artifacts that preclude accurate slide evaluation.

Immunohistochemistry Services

Additional diagnostic tests for infectious agents and cell markers are available by immunohistochemistry and may be recommended by the case pathologist. IHC is performed on fixed tissue samples procured from diagnostic necropsies or mail-in biopsies with the exception of ear notches from cattle which can be submitted in formalin for bovine viral diarrhea virus testing by this method.

B. Bacteriology

The Rollins Bacteriology Section provides isolation and identification of a wide variety of aerobic and anaerobic bacteria, fungi and mycoplasmas. In addition, parasitology services are offered and include fecal flotation, centrifugation-sucrose, modified McMaster's, *Tritrichomonas foetus* culture and fluorescent antibody testing for *Cryptosporidium* and *Giardia*.

Bacteriology Submission Guidelines

The value of bacterial culture is directly dependent upon the quality of the specimen. Improperly collected or transported specimens may lead to erroneous culture results. The following general guidelines should be considered:

1. Collect specimens aseptically from an appropriate site, during the acute stage of disease, and prior to antimicrobial therapy.
2. Submit generous volumes, if possible.
3. Submit a separate sample for each discipline/department requested (bacteriology, cytology, etc.)
4. Avoid swab specimens, if possible. Swabs are easily contaminated and most often do not provide a sufficient volume of specimen for culture.
5. Place specimens in an appropriate transport media to maintain a buffered and non-nutritive environment and to prevent desiccation. Make sure the transport media has not passed the expiration date set by the manufacturer.
6. Place multiple tissue specimens in separate containers to prevent cross-contamination.
7. Use an indelible marker to label specimens with the location (tissue) and animal species of origin.
8. Generally, maintain specimens at refrigeration temperature immediately after collection and send to the laboratory with cold packs.
9. Package specimens carefully to ensure there will be no leakage or breakage in transit.
10. Always indicate the test request.
11. Antimicrobial susceptibility testing (AST) is available for aerobic bacteria only either by Kirby disk diffusion (default) or Microbroth dilution MIC method. Please note specific AST test request on the submission form.
12. Susceptibility tests are conducted in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines and are performed on isolates deemed to be significant. Susceptibilities may not be performed on isolates that represent normal flora or common contaminants, as it will not provide useful information and may lead to inappropriate antimicrobial therapy. We are unable to perform susceptibilities on fastidious organisms for which there are no recommended guidelines or interpretive criteria.
13. Different microbiologic culture procedures require different times for completion. Refer to Test Schedule for further details.

BACTERIOLOGY SPECIMEN SELECTION & TRANSPORT GUIDELINES

CONDITION	POSSIBLE ETIOLOGIC AGENTS	SPECIMEN(S)	COLLECTION/ TRANSPORT
Abortion	*Brucella* species (spp.) *Campylobacter* spp. *Leptospira* spp *Listeria monocytogenes* Many others	Fetus: fresh, intact fetus or lung, liver, brain, kidney, stomach contents. Fetal thoracic fluid. Dam: Placenta with cotyledons, vaginal discharge or swab, cervical mucus.	Refrigerated.
Abortion, mycotic	*Aspergillus* spp. zygomycetes	Placenta with cotyledons. Fetal stomach contents and/ or skin lesions.	Refrigerated.
Abscess	*Actinomyces* spp. Anaerobes *Trueperella pyogenes* *Pasteurella multocida* Staphylococci Streptococci	Exudate or swab in transport medium. Biopsy in sterile saline.	Refrigerated with exception of anaerobes, which needs to be at room temperature.
Actinomycosis or actinobacillosis	*Actinomyces bovis* *Actinobacillus lignieresii*	Exudate with granules or lesion. Swab of abscess material in transport medium or collected in syringe w/o needle.	Refrigerated.
Anaerobic infections	Clostridia *Bacteroides* spp. *Fusobacterium* spp. *Prevotella* spp. *Porphyromonas* spp.	Large piece of affected tissue. Exudates/ swabs need to be in anaerobic transport medium. Ligated segments of affected intestine.	Room temperature.

CONDITION	POSSIBLE ETIOLOGIC AGENTS	SPECIMEN(S)	COLLECTION/ TRANSPORT
Arthritis	*Streptococci* *Trueperella pyogenes* *Haemophilus* species *Erysipelothrix rhusiopathiae* *Mycoplasma* species *Staphylococcus aureus*	Entire affected joint from smaller animals. Synovial tissue in sterile saline. Joint swab in transport medium. Joint fluid collected in blood culture medium or in sterile syringe w/o needle.	Refrigerated. Blood culture medium needs to be at room temperature.
Atrophic rhinitis of swine	*Bordetella bronchiseptica* and/or *Pasteurella multocida*	Ante-mortem: Deep swab of nasal cavity, placed in sterile saline. Tonsil swab or biopsy in sterile saline. Post-mortem: send entire snout or turbinate swabs in an aerobic transport medium such as Stuart's.	Refrigerated.
Bartonellosis	*Bartonella* spp.	Blood in blood transport medium. Heart valves, lymph node aspirates in sterile container.	Blood culture medium at room temperature. Tissues refrigerated or frozen.
Black leg, gangrene	*Clostridium chauvoei, novyi, perfringens, septicum, sordellii*	Fresh piece of muscle with lesion. Impression smear slides from affected tissue for fluorescent antibody test.	Room temperature.

CONDITION	POSSIBLE ETIOLOGIC AGENTS	SPECIMEN(S)	COLLECTION/ TRANSPORT
Botulism	*Clostridium botulinum*	Food suspected of containing toxin. Ligated sections of fresh intestine. Large section of liver. Serum. Samples may be forwarded to a reference lab.	Refrigerated tissues. Frozen serum.
Bovine respiratory disease	*Histophilus somni* *Pasteurella multocida* *Mannheimia haemolytica* *Mycoplasma* species	Ante-mortem: Transtracheal aspirate in sterile container, or deep nasal swab. Post-mortem: Lung at demarcation between normal and affected tissue.	Refrigerated.
Brucellosis reactor	*Brucella* spp.	Ante-mortem: Milk, vaginal secretions, semen, blood cultures, hygroma (fluid from a swollen joint). Post-mortem: Head, mammary and genital lymph nodes, spleen, reproductive organs.	Refrigerated. Must be received with animal identification tag.

CONDITION	POSSIBLE ETIOLOGIC AGENTS	SPECIMEN(S)	COLLECTION/ TRANSPORT
Campylobacteriosis (bovine and ovine)	*Campylobacter fetus* ss. *venerealis*, or ss. *fetus Campylobacter jejuni*	Aborted fetus, or fetal lung, liver, stomach contents; placenta. Male: preputial mucus or secretions, semen. Female: cervical or vaginal mucus. Mucus specimens or semen must be in special transport medium such as fluid thioglycollate, Amie's with charcoal or Clark's.	Refrigerated. Must be received within 2448 hours of collection. Frozen tissue specimens are also acceptable.
Campylobacteriosis (canine and equine)	*Campylobacter jejuni*	Fresh rectal/fecal swabs, fresh diarrheic feces collected in Cary-Blair or other medium suitable for maintaining *Campylobacterium* viability.	Refrigerated. Delivered within 24-48 hours of collection. Frozen specimens also acceptable.
Caseous lymphadenitis	*Corynebacterium pseudotuberculosis*	Affected lymph node; abscesses, exudates in sterile container; swabs in aerobic transport medium.	Refrigerated.
Colibacillosis	*Escherichia coli*	Affected portions of intestines, fresh feces, or fecal swabs in aerobic transport medium.	Refrigerated.
Cystitis	*Escherichia coli* *Proteus* species *Enterococcus* species *Staphylococcus aureus*	5-10 ml of fresh urine in Urine culture tubes or sterile container. Bladder swabs in aerobic transport medium.	Refrigerated. Ice packs preferable. Delivered within 24 hours of collection

CONDITION	POSSIBLE ETIOLOGIC AGENTS	SPECIMEN(S)	COLLECTION/ TRANSPORT
Dermatophytosis (ringworm)	*Microsporum* and *Trichophyton* species	Skin scrapings or hairs. Swabs unsuitable.	Room temperature.
Dermatophilosis ("rain rot")	*Dermatophilus congolensis*	Scabs and crusts in sterile container. Skin biopsy in sterile saline.	Room temperature.
Enterotoxemia	*Clostridium perfringens*	Several ounces of fresh intestinal contents in sterile container.	Refrigerated. Frozen specimen preferable.
Enteritis (diarrhea)	*Escherichia coli* *Salmonella enterica* Others	Fresh, diarrheic feces in sterile container. Tied-off loops of affected intestine.	Refrigerated.
Erysipelas	*Erysipelothrix rhusiopathiae*	Acute form: Heart blood, kidney, spleen, liver. Arthritic and cardiac form: joints and heart valves (swabs in aerobic transport medium).	Refrigerated.
Greasy pig disease, exudative epidermitis	*Staphylococcus hyicus Streptococcus* species	Skin scrapings in a sterile container. Skin swabs in aerobic transport medium.	Refrigerated.
Glässers disease ("Hps")	*Haemophilus parasuis*	Brain, heart, lung, and intact, swollen joints or other organs with fibrinous coating. Swabs are not acceptable.	Refrigerated.
Keratoconjunctivitis, bovine	*Moraxella species*	Conjunctival swabs in aerobic transport medium.	Refrigerated. Must arrive at lab within 24 hours of collection.
Interdigital dermatitis ("Footrot")	*Dichelobacter nodosus* *Fusobacterium necrophorum*	Surgical biopsy of affected tissue in anaerobic transport medium	Room temperature

CONDITION	POSSIBLE ETIOLOGIC AGENTS	SPECIMEN(S)	COLLECTION/ TRANSPORT
Listeriosis	*Listeria monocytogenes*	Neural form: Brain stem. Visceral form: liver. Abortion form: placenta and fetus or fetal stomach contents. Feed samples.	Refrigerated.
Mastitis	*Staphylococcus* species *Streptococcus* species *Mycoplasma* species Coliforms, many others	Five to ten mls of milk collected in a sterile, leakproof container.	Refrigerated or frozen. Samples may be frozen up to 2 weeks.
Meningitis	*Streptococcus* species *Streptococcus suis* *Histophilus somni* *Cryptococcus neoformans*	Ante-mortem: Aseptically collected cerebrospinal fluid in blood culture medium. Post-mortem: Brain, meningeal swabs in aerobic transport medium	Blood culture medium at room temperature. Refrigerated tissues.
Mycobacteriosis (other than Johne's disease or tuberculosis)	Rapidly and slow growing *Mycobacterium* spp.	Skin lesions, draining tract swabs, biopsies, tissues with granulomatous lesions, feces, body fluids.	Refrigerated.

CONDITION	POSSIBLE ETIOLOGIC AGENTS	SPECIMEN(S)	COLLECTION/ TRANSPORT
Mycoplasmosis	*Mycoplasma* species	Ante-mortem: Tracheal exudates and aspirates, milk, choanal or conjunctival swabs, joint fluid. Swabs must be in appropriate transport medium. Post-mortem: lung tissue with bronchi, trachea, sinuses, air sacs, intact affected joint.	Refrigerated and delivered within 48 hours of collection. Frozen tissue specimens are suitable.
Nocardiosis	*Nocardia asteroides* and other species	Biopsy in sterile container. Aspirates, exudates (to include granules). Transtracheal wash in sterile container.	Room temperature.
Otitis externa	*Proteus* species *Pseudomonas* species *Staphylococcus* species *Streptococcus* species Many other bacteria Yeasts (*Malessezia*)	Ear swab placed in aerobic transport medium.	Refrigerated.
		Impression smear slide.	Room temperature.
Pleuropneumonia of swine ("APP")	*Actinobacillus pleuropneumoniae*	Portion of affected lung or other tissues with lesions.	Refrigerated.
Pseudomembranous colitis	*Clostridium difficile*	Affected portion of colon, colon contents in anaerobic transport medium.	Refrigerated/frozen.

CONDITION	POSSIBLE ETIOLOGIC AGENTS	SPECIMEN(S)	COLLECTION/ TRANSPORT
Pyelonephritis, bovine	*Corynebacterium renale*	Ante-mortem: Midstream sample of urine in a sterile container. Post-mortem: Portion of affected kidney, ureter, bladder and urethra.	Refrigerated.
Rhodococcal pneumonia of foals	*Rhodococcus*	Ante-mortem: Transtracheal wash in sterile container. Post-mortem: Fresh lung with lesions and respiratory lymph nodes.	Refrigerated.
Salmonellosis	*Salmonella enterica*	Ante-mortem: Fecal swabs or 1-5 gm of feces from diarrheic animals. A minimum of 3 specimens collected on 3 sequential days is preferred. Post-mortem: Intestines, liver, gall bladder, spleen, lung, lymph nodes, bone marrow, feces, intestinal contents.	Refrigerated.
Septicemia	Staphylococci Streptococci Enteric bacteria others	Ante-mortem: Blood collected aseptically during a febrile spike in a blood culture system.	Room temperature.
		Post-mortem: Heart blood, bone marrow, spleen, liver, lungs.	Refrigerated

CONDITION	POSSIBLE ETIOLOGIC AGENTS	SPECIMEN(S)	COLLECTION/ TRANSPORT
Sporotrichosis	*Sporothrix schenckii*	Biopsy material from unopened skin nodules or scrapings from skin ulcers, placed in sterile containers with saline.	Refrigerated.
Strangles, equine	*Streptococcus equi* ss. *equi*	Abscess material on swab in aerobic transport medium or in syringe w/o needle.	Refrigerated.
Swine dysentery/ spirochetal colitis	*Brachyspira hyodysenteriae* *Brachyspira pilosicoli* or other species	Ante-mortem: Fecal or rectal swabs in anaerobic transport medium. Post-mortem: Spiral colon, colonic scrapings, feces.	Refrigerated.
Systemic fungal infections (blastomycosis, histoplasmosis, coccidioidomycosis, cryptococcosis)	*Blastomyces dermatitidis* *Coccidioides immitis* and *posadasii* *Histoplasma capsulatum* *Cryptococci*	Exudates from draining tracts/lesions; transtracheal washes; CSF; ocular fluid; prostatic fluid; urine; lymph node aspirates/ biopsies; bone; other tissues with lesions. All placed in sterile container with saline.	Refrigerated with exception of CSF, which should be room temperature.
Tuberculosis	*Mycobacterium* spp.	Affected portions of lung, liver, spleen, lymph nodes (mediastinal, cranial, bronchial, portal). Bone marrow. Other tissues/organs with granulomatous lesions.	Refrigerated. Cultures not performed by NCVDLS. Tissues forwarded to NVSL.

CONDITION	POSSIBLE ETIOLOGIC AGENTS	SPECIMEN(S)	COLLECTION/ TRANSPORT
Tularemia	*Francisella tularensis*	Heart blood, liver, spleen, bone marrow, or other organs with white necrotic foci.	Refrigerated. Must state on submission form "tularemia suspect".

C. Bacterial Serology

Serum samples from various animal species are tested for antibodies to a variety of infectious agents. Regulatory testing for brucellosis, avian mycoplasmosis and anaplasmosis are also performed.

Blood samples deteriorate quickly. Please package them so they will stay cool during transit. Whenever possible, serum samples should be poured off the clot and shipped cold or frozen. If paired samples are to be tested, the acute serum should be held frozen and sent to the laboratory along with the convalescent serum sample for parallel testing.

Instructions for collecting poultry blood samples:

1. Collect at least 500 µl of blood, but do not overfill the blood tube. The tube should be about half full of blood.
2. Place the blood tubes in the cardboard tube box and tilt the box approximately 30 degrees. After collection, the samples should be transferred to an ambient temperature environment (≈70°F) as soon as possible.
3. Keeping the box tilted, samples should be allowed to clot at ambient temperatures for approximately 1 hour. Do not refrigerate the samples before they have had time to clot.
4. Once the samples have clotted, refrigerate them until they can be brought to the lab.
5. Samples should be submitted to the lab within 24 hours of collection. If this is not possible, keep the samples refrigerated until they can be delivered to the lab.
6. When refrigerated, samples should never be held for more than 4 days prior to delivery to the lab. If long-term storage is required, the serum should be collected and frozen.
7. If you wish to freeze the sample, remove the serum from the clot and transfer it to a separate tube with a cap. Do not freeze the serum while still on the clot.

D. Molecular Diagnostics

Nucleic acids (DNA and/or RNA) are present in all living things (animal, man, bacterium, virus) and are specific to each species. The basic molecular diagnostic test method employed for nucleic acid detection is the "PCR" or polymerase chain reaction assay. PCR is a highly sensitive and specific method used to amplify nucleic acids for diagnostic purposes. The ability to specifically detect DNA/RNA target sequences of a microorganism in a sample is direct evidence of its presence in that specimen. Proper collection and transport of specimens are essential to ensure

reliable test results. Nucleic acid integrity must be maintained throughout these processes.

General Requirements for Molecular Diagnostics

Specimen containers should be tightly sealed and labeled as to animal identity and date of collection using an indelible marker. Containers should be clean on the outside (i.e. no fecal material, blood, or dirt), as unclean containers compromise a laboratory's ability to prevent contamination of the lab environment and other specimens. Sample tubes should not be filled to the top because these tubes may expand during storage or shipment resulting in sample leakage and contamination. If swabs are to be used, they should be made of Dacron, as materials in other types of swabs can be inhibitory to PCR. The number and type of swabs collected per tube should be indicated on the submittal form.

Note: Wooden handled swabs with cotton tips are not acceptable for PCR.

Avian Influenza

Avian influenza is an acute viral disease of birds that is often characterized by high mortality in all age groups. Tracheal or oropharyngeal swabs are the specimens of choice. These must be submitted in either Brain Heart Infusion (BHI) broth or a commercial transport medium (Remel MicroTest M4 Transport Medium, up to 5 swabs may be pooled in 3ml tube of medium, up to 11 swabs may be pooled in 5.5ml tube of medium. Note: 5.0 ml BHI is acceptable when 5.5 ml BHI is not available). Dry swabs are unsuitable for testing. Samples should be shipped with cold packs for next day delivery.

Avian Reovirus real-time RT-PCR

Avian reoviruses are widespread in domestic and wild avian species and are associated with a wide range of clinical symptoms. However, most ARV infections are seen in broilers, broiler breeders, and turkeys at various ages displaying severe viral arthritis/tendosynovitis. These symptoms cause serious economic losses, and the need for an accurate diagnostic tool is needed for disease control measures.

Acceptable samples: Tendons, tendon swabs, virus isolates.

Bovine Viral Diarrhea Virus

Bovine viral diarrhea virus (BVDV) is an economically important disease of ruminants that causes respiratory and reproductive problems. The laboratory has multiple methods to detect BVD virus. The tests include virus isolation, immunohistochemistry (IHC), PCR and antigen capture ELISA. The particular tests performed depend on the clinical presentation and/or purpose of testing. Some tests are more efficient for whole-herd testing for identifying BVDV persistently infected animals, including camelids. Please see the fee schedule for specific test charges and the test schedule for specimen requirements. Specifically for PCR, serum is the preferred sample for cattle. Sera from up to five cattle can be pooled for testing purposes. Call the laboratory for assistance in choosing any of the available options.

Clostridium perfringens genotyping

Genotyping is performed on isolates that have been recovered from the intestines of affected animals. Organism purity and identity will be verified prior to testing. Cultures should be shipped to the laboratory with cold packs.

E. coli genotyping

Disease associated with *E. coli* infections relies on the differentiation of pathogenic from non-

pathogenic strains. To cause disease, *E. coli strains* possess virulence factors such as enterotoxins and adhesins. The former are associated with secretory diarrhea, while the latter are responsible for promoting attachment to and colonization of intestinal cells. The multiplex PCR assay used at this laboratory has been developed to detect the presence of genes for the most common enterotoxins (LT, STa, STb) and adhesins (K88, K99, 987P, F41, F18) associated with disease in bovine and porcine species.

Equine Herpes Virus

Equine herpesviruses (EHV) are common in horse population worldwide. Multiple strains have been identified and cause several equid diseases. Two closely related viruses, EHV-1 and EHV-4 are specifically addressed at Rollins. Both are members of the genus *Varicellovirus,* subfamily *Alphaherpesvirinae,* family *Herpesviridae*. EHV-1 and EHV-4 are highly contagious, and disease caused by either virus is commonly referred to as equine rhinopneumonitis. Symptoms include fever, loss of appetite, depression, and nasal discharge. EHV-1, and occasionally EHV-4, may also cause abortion and neonatal death, and is sometimes referred to as equine abortion virus. A neurologic form of EHV-1 is now recognized as the causative agent of equine herpesvirus myeloencephalopathy (EHM). A specific genome mutation in EHV-1 links it to EHM. Neuropathogenic strains have been associated with large outbreaks of EHM, while the non-mutant strains can cause neurologic disease in individuals but, to date, are not associated with large outbreaks of EHM. Almost all horses are exposed to EHV and the virus remains latent in the horse throughout it life. After the primary respiratory infection, reinfection or recrudescence may occur with few clinical signs. Rollins uses three real-time polymerase chain reaction (PCR) tests. The first two assays are specific for EHV-1 and EHV-4 respectively, target the glycoprotein B genome region of each virus. The third assay is specific to EHV-1 and further identifies a single nucleotide polymorphism (SNP) within the DNA polymerase genome open reading frame 30 (ORF 30). The SNP is useful in distinguishing horses at greater risk for the neurovalent pathotype of EHV-1. The PCR technique will detect viral nucleic acid but the test does not determine if the infectious virus is present.

Exotic Newcastle Disease – ***Please contact laboratory prior to submission***.

Exotic Newcastle disease (END), previously known as velogenic viscerotropic Newcastle disease, is a highly contagious and fatal viral disease affecting all avian species. Tracheal or oropharyngeal swabs must be submitted in Brain Heart Infusion (BHI) broth or Remel MicroTest M4 Transport Medium. Up to 5 swabs may be pooled in 3ml tube of medium, up to 11 swabs may be pooled in 5.5ml tube of medium (Note: 5.0 ml BHI is acceptable when 5.5 ml BHI is not available). Dry swabs are unsuitable for testing. Samples should be shipped with cold packs for next day delivery.

Infectious Bronchitis

Infectious Bronchitis is a highly infectious disease of the upper-respiratory tract of chickens, which can also affect the kidneys (nephrogenic strains) and reproductive tract. It is an economically important disease to the poultry industry because of the high morbidity and condemnation losses at processing due to air sacculitis as a result of viral infection. Tracheal swabs in a closed tube or Ziploc® bag, pooled tracheal swabs (three per pool) placed in 3 ml BHI medium, or fresh tracheas in a Ziploc® bag are acceptable samples for testing. Samples should be stored in the refrigerator until they can be shipped to the lab with cold packs for overnight delivery.

Infectious Laryngotracheitis

Infectious Laryngotracheitis (ILT) is an upper-respiratory disease of poultry caused by an alphaherpesvirus. The disease is extremely contagious and is spread easily by aerosol from water vaccinated breeder flocks, and by contamination of equipment, people, litter, and other objects. Tracheal swabs in a closed tube or Ziploc® bag, pooled tracheal swabs (three per pool) placed in BHI medium, or fresh tracheas in a Ziploc® bag are acceptable samples for testing. Samples should be stored in the refrigerator until they can be shipped to the lab with cold packs for overnight delivery. **For same day results, specimens should be received by 10:00 AM.**

Lawsonia intracellularis

Lawsonia intracellularis is the causative agent of proliferative enteropathy (PE) or ileitis in swine and other domestic animals. The bacterium causes proliferation of intestinal cells, resulting in enteric disease or even death. The disease is responsible for serious economic losses in swine production worldwide.

Fresh, affected segments of intestines or fecal samples from animals suspected of having PE should be submitted. These should be stored in the freezer and shipped to the laboratory with cold packs or dry ice.

Please note: *Lawsonia* organisms are shed intermittently in the feces. Tests on multiple samples may be necessary to detect the presence of this agent.

Mycoplasma gallisepticum and *Mycoplasma synoviae*

Mycoplasmosis is a widespread disease affecting poultry production worldwide. *Mycoplasma gallisepticum* is one of the etiological agents of chronic respiratory disease in hens and infectious sinusitis in turkeys. *Mycoplasma synoviae* is responsible for a subclinical infection of the respiratory tract and causes synovitis.

Tracheal samples should be obtained by swabbing the trachea of chickens or turkeys with clinical signs of mycoplasmosis. Swabs should be placed in tubes containing 3 ml of BHI broth. Samples should be stored on ice, shipped with ice or cold packs and delivered to the laboratory within 24 hours of collection. For pooled samples, up to five swabs may be placed in tubes of transport medium.

Samples accepted for testing are whole blood collected in EDTA or Heparin tubes, swabs, or tissue. Samples should be stored in the refrigerator until they can be shipped to the lab with cold packs for overnight delivery. **For same day results, specimens should be received by the laboratory by 10:00 AM.**

Porcine Circovirus 2/3 (PCV 2/3) real-time PCR

PCV2 is strongly associated with post weaning multi-systemic wasting syndrome (PMWS), also known as porcine circovirus type 2 systemic disease (PCV2-SD) and porcine circovirus type 2 subclinical infection (PCV2-SI). PCV2 has become widely distributed in most developed swine industries around the world. While most swine are infected with PCV2, only a smaller portion of pigs show signs of the disease. PCV3 has been associated with signs similar to PCV2, however, many infections seem to be subclinical. PCV3 has been associated with neurological disease, reproductive failure, respiratory disease, enteric disease, and porcine dermatitis and nephropathy syndrome (PDNS). Epidemiological studies have found that infection is widespread, with prevalence up to 100%. A few studies have shown that prevalence is highest in piglets/weaners and decreases with age, but PCV3 has been detected in pigs up to 23 weeks of age.

Acceptable samples: Oral fluid, serum, blood, body cavity fluid, swabs, urine, feces, and tissue homogenates.

Porcine Reproductive and Respiratory Syndrome

This widespread disease is associated with epidemic abortions, infertility, and acute pneumonia. The PCR assay is able to discriminate between US and European virus and it is a multiplex PCR assay.

Fresh lung tissue, fetal thymus, fetal thoracic fluid, serum, oral fluid, or lung lavage fluid should be submitted for testing. These should be stored overnight in the refrigerator and mailed with cold packs for next day delivery.

Swine Enteric Coronavirus Disease

Swine Enteric Coronavirus Disease consists of Transmissible Gastroenteritis (TGE), Porcine Epidemic Diarrhea Virus (PEDv), and Swine delta Coronavirus (SDCoV). Both PEDv and SDCoV have recently spread throughout the swine industry in the United States. All three viruses (TGE, PEDv, and SDCoV) produce similar clinical signs of vomiting, severe diarrhea, with high morbidity and variable mortality. Mortality in pigs up to two weeks of age can routinely reach 100%. Samples of intestine, intestinal swabs, feces, environmental swabs or fluids, and/or oral fluid should be submitted chilled in secure containers for PCR testing.

Effective June 5, 2014, the US Secretary of Agriculture by Federal Order requires producers, veterinarians, and veterinary diagnostic laboratories to report new cases of SECD (PEDv and SDCv) to the appropriate state and/or federal animal health officials. Please call the Rollins Laboratory regarding testing questions.

Swine Influenza

Swine influenzavirus is an acute, highly contagious respiratory disease. Lung tissue, bronchial or nasal swabs, or lung should be submitted for testing. Samples should be kept refrigerated and mailed with cold packs for next day delivery.

Turkey Coronavirus

Turkey coronavirus is an important etiological cause of diarrhea in young poults and clinical signs are most severe and mortality highest in poults up to one month of age. The laboratory has a PCR test available for use on intestine and intestinal swabs (up to five samples can be pooled for testing) on young poults.

West Nile

West Nile virus was first detected in the Western Hemisphere in 1999 and has since rapidly spread across the North American continent. This mosquito-transmitted virus causes inflammation or swelling of the brain and spinal cord in horses and humans. Brain stem, cerebellum, and cerebrum from clinically affected animals should be submitted for testing. These sections should be placed in individual bags, labeled, and stored in the refrigerator until they can be shipped to the lab with cold packs for overnight delivery.

E. Parasitology

Limited parasitology services are available at NCVDLS. Please refer to the test schedule for where tests are performed.

These services are NOT available: heartworm check, Baermann floatation.

Fecal Examination

Fecal samples must be fresh for accurate results. If specimens have been in the environment for several hours or days, many fragile protozoan parasites may have died or disintegrated. Nematode eggs often hatch rendering them more difficult to identify. Also, free-living soil nematode, fly larvae, or mites may invade the specimen on the ground and cause difficulty in the differentiation of hatched parasite larvae from non-pathogenic species.

Specimens should be submitted in individual sealed containers; plastic specimen cups, Whirl-pack ® or zip-lock bags are recommended. These should be labeled with the animal identification and date of collection using an indelible marker. **Please do NOT send feces in OB sleeves or exam gloves.** A minimum of 5 grams of feces is necessary for accurate results. If samples are greater than 2 hours old, they should be held at refrigerator temperatures and shipped to the laboratory packed in ice or other coolant. Results are usually available within 48 hours of receipt.

Fecal Floatation

Zinc sulfate solution is used. This is a qualitative test and results are reported as few, moderate or many.

Quantitative Floatations (EPG)

Modified McMaster's: Zinc sulfate solution is used. For ruminant and equine samples, only trichostrongyle/strongyle–type ova are counted. For camelid samples, all ova types are counted.

Centrifugation Sucrose: Sheather's sugar solution is used. This is the recommended test for camelids when looking for *Eimeria macusaniensis.* All ova types are counted.

Cryptosporidium and *Giardia*

The Rollins Laboratory offers a direct immunofluorescence assay for the detection of cryptosporidium oocysts and *Giardia* cysts from stool specimens. Specimens should be submitted in 10% formalin (1:1 ratio) or SAF fixative (sodium acetate formalin). Specimens should **not** be preserved in polyvinyl alcohol.

Parasite Identification

Parasite identification is performed at NCSU/CVM through a cooperative agreement. Specimens should be sent to the Rollins Laboratory. Helminths and external arthropod parasites should be submitted in leak-proof vials or small jars containing 70% ethanol or 10% formalin and labeled appropriately. When mites are suspected, skin scrapings should be placed in glycerin in a tightly sealed vial. Results generally take 7 days.

Tritrichomonas species

Clinical studies have demonstrated the superiority of the In-Pouch® proprietary system for the collection, transport and cultivation of *T. foetus* in cattle, *T. gallinae* in birds, and intestinal trichomonads in felines. Media are available from the Rollins Laboratory on a limited basis or may be purchased directly from the manufacturer (Bio-Med Diagnostics, Inc., 1388 Antelope Rd., White City, OR 97503) by calling 800-964-6466. Specimens should be collected per the manufacturer's instructions, held at 15-37°C and shipped to the laboratory as soon as possible. Allow a minimum of 7 days for test completion.

F. Virology

The Virology Laboratory Section provides virus isolation and identification testing for mammalian and avian viruses. The laboratory uses cell culture and embryonating eggs to isolate virus. Specific viruses are identified by fluorescent antibody (FA), virus neutralization (VN, SN), agar gel immunodiffusion (AGID), hemagglutination (HA), hemagglutination-inhibition (HI) and/or polymerase chain reaction (PCR) tests. The laboratory utilizes electron microscopy and antigen-capture to assist in identifying viruses by direct examination.
The Virology Section also conducts tests for specific antibody by virus neutralization (VN/SN), enzyme-linked immunosorbent assay (ELISA), agar gel immunodiffusion (AGID), hemagglutination-inhibition (HI) for economically important mammalian and avian viruses.
As we are continuously adapting our testing capabilities to best fit our clients' needs, feel free to contact the laboratory directly if you have questions about the availability of testing.

General Requirements for Virology Submissions

The quality of the sample submitted for testing will directly impact the reliability of test results. Samples should be collected and kept cold prior to and during shipping. When possible, samples should be sent to the lab within 24hrs of collection. Overnight shipment through FedEx or UPS is recommended.

Equine Infectious Anemia (EIA)/Coggins Test

1. All testing is done in accordance with USDA rules and regulations (9CFR75.4, VS Memo 555.7 and 555.16).
2. Only a licensed and accredited Veterinarian may submit samples for testing.
3. Samples must be sent with a completed VS10-11 form. VS10-11 forms are available from the USDA.
4. The Rollins laboratory participates in electronic submission through Global Vet Link (GVL) and the USDA Veterinary Streamline Processing Service (VSPS).
5. Samples should be clearly labeled to match paperwork. Incomplete paperwork or mismatched samples may result in delayed testing.
6. The Veterinarian accreditation code or license number must be listed on all submission forms. Failure to include a valid accreditation or license number may delay reporting of final results.
7. Routine testing is done by AGID. Samples are typically set up once per day, usually in the afternoon. The AGID test requires a 24hr incubation period. Samples received before 3pm will be processed and results will be available by 5pm the next business day. **Samples received after 3pm will be tested the next business day.**
8. Rush testing is available for an additional fee ($15.00 per sample, plus cost of testing). Rush testing covers all testing where clients require results within a 24-hr. period. Contact the lab for availability.
9. Clients wishing to test for import/export purposes are encouraged to contact the National Veterinary Service Laboratory (NVSL) in Ames, IA.

Viral Serology

1. To ensure sufficient serum for mammalian testing, collect at least one red-top tube (≈3-5ml) per sample and allow the blood to clot for 1 hour or so and then refrigerate until shipped. Clients with access to a centrifuge can spin blood, collect the serum or use a serum separator tubes for best results.
2. For avian serology, submit at least 1-2ml of whole blood (0.2 to 0.5ml of serum) when possible. If requesting AIV ELISA, at least 0.5ml of serum is required. If requesting multiple tests, ensure to submit a sufficient volume of sample (e.g., 0.5ml of serum for AI and 0.5ml for titers).
3. All samples should be clearly labeled and match submission paperwork. Clearly indicate which tests are needed. When samples from two farms are in one box, note that information in the paperwork and also on the top and sides of the box.
4. Sample testing turn-around times vary with each test and are dependent upon number of samples received. See testing guide for additional details about individual tests.

Virus Isolation

1. Collect tissue samples aseptically from appropriate sites. Clearly mark all paperwork and samples. Samples collected during acute phases of illness are best. Contact the laboratory with questions about appropriate samples and collection.
2. Samples sent to the lab within 24-48hrs can be refrigerated and sent with ice packs. If shipping will be delayed longer than 48hrs, then samples should be frozen and shipped on dry ice. Keep samples cold at all times.
3. Swabs should be submitted in a viral transport media when possible. Contact the laboratory for additional information.
4. For liquid samples, submit at least 10ml when possible.
5. Virus isolation may require several passages and follow-up testing for positive identification. This process may take several weeks.

G. Toxicology/Chemistry

This service was closed at NCVDLS in 2007 but is being partially re-established as of 2022. Three tests are currently offered at Rollins. Calculi Identification by FTIR determines percent composition of biologically generated stones to assist with prevention of recurrence. Toxicology Evaluation (visual/microscopic) can be applied to rumen content, stomach content, forage, or hay for possible toxic plant material or visually identifiable foreign object contamination to help direct further testing. Toxicology Evaluation can also be used to check for blister beetle fragments in equine stomach content or cecal content, or for the beetles in hay; for lead fragments in reticulum content; or for cyanobacteria (blue-green algae) in water samples. Toxic Plant Identification (visual/microscopic) is also available.

Most Analytical Toxicology testing is outsourced to the Pennsylvania Animal Diagnostic Laboratory System (PADLS) through a Memorandum of Understanding. Clients will be charged a shipping and handling fee of $20 by NCVDLS. PADLS will bill them directly for services rendered. Test services that are available may be found at: http://www.padls.org.

H. Outsource/Referrals

NCVDLS outsources work if it does not have the capability to perform an analysis. Outsourced requests are only submitted to laboratories that demonstrate competency for the work performed unless the client specifically requests that a particular laboratory be used. Samples outsourced to other laboratories for analysis are charged at the rate of the subcontracting laboratory or are billed directly to the client. A handling fee, which includes a routine shipping charge, is listed in the General Laboratory Policies in the current Fee Schedule.

V. Test Schedule

NCVDLS realizes that our clients expect test results in a timely manner. To meet these expectations, we have developed turnaround time goals which are the average amount of time it takes to perform a test and generate a result. Although we will make every effort to adhere to these schedules, the availability of test results will be impacted by laboratory workload, holidays, test reagent/supply availability, specimen quality, specimen arrival time, inclement weather, or staffing issues.

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Acid fast stain	Rollins/Bacti	Fresh affected tissue, feces	Ice pack	M-F	1-2 days	For detection of acid-fast bacilli.
Adenovirus	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
Adenovirus 127 (HI)	Rollins/Viro	Serum	Ice pack	Volume dependent	2-4 days	NVSL
Aerobic culture/ susceptibility	Rollins/Bacti	Affected tissues, fluids, swabs	Ice pack	M-F	3-5 days	Leak-proof sterile containers for tissues and fluids. Aerobic transport medium for swabs.
Anaerobic culture	Rollins/Bacti	Affected tissues, fluids, swabs	None	M-F	7-14 days	Leak-proof sterile containers for tissues. Anaerobic transport medium for fluids and swabs. Ship unrefrigerated by express delivery.
Anaplasmosis (cELISA)	Rollins/Sero	Serum	Ice pack	M-F	3-5 days	Bovine species only.

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Avian Encephalomyelitis Virus (ELISA)	Rollins/Viro	Serum	Ice pack	Volume dependent	1 day	IDEXX kit
Avian Influenza (AGID)	Rollins/Viro Griffin Northwestern	Serum/Egg	Ice pack	M-Thur	1-2 days	For NPIP testing, refer to current auxiliary provisions. NVSL test antigen
Avian Influenza (ELISA)	Rollins/Viro Griffin	Serum	Ice pack	M-W-F	1-2 days	For NPIP testing, refer to current auxiliary provisions. At least 0.5ml of serum is required. For detecting antibodies in chickens. Zoetis kit and IDEXX kit
Avian Influenza H5 (RRTPCR)	Rollins/Mol	Tracheal or oropharyngeal swabs, see comments	Ice pack	Tues, W, and F	Same day if received by 10:30 am, or with prior notification	Collect swabs in either Brain Heart Infusion (BHI) broth or a commercial transport medium, such as Remel M4. Up to 5 swabs per 3ml tube; up to 11 swabs per 5.5ml tube of medium.
Avian Influenza H7 (RRTPCR)						
Avian Influenza (Matrix RRT-PCR)						
Avian Influenza-multiple species (ELISA)	Rollins/Viro	Serum	Ice pack	Volume dependent	1-2 days	For NPIP testing, refer to current auxiliary provisions. For detecting antibodies in chicken, turkey, duck, ostrich, and goose sera. IDEXX kit
Avian Influenza (Antigen Capture ELISA)	Rollins/Viro Griffin Northwestern Western	Tracheal or oropharyngeal swabs	Ice pack	M-F	1 day	Collect up to 5 swabs in 3ml tube of BHI broth
Avian Paramyxovirus-1 (APMV-1) (ELISA)	Rollins/Viro	Serum	Ice pack	Volume dependent	1-2 days	IDEXX kit
Avian Paramyxovirus-1 (APMV-1) (ELISA), Turkey	Rollins/Viro	Serum	Ice pack	Volume dependent	1-2 days	IDEXX kit

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Avian Paramyxovirus-1 (APMV-1) (HI)	Rollins/Viro	Serum	Ice pack	Volume dependent	1-2 days	Charles River test antigen
Avian Paramyxovirus-1 (APMV-1) (Matrix RRTPCR)	Rollins/Mol	Tracheal or oropharyngeal swabs, see comments	Ice pack	Tues, W, and F	Same day if received by 10:30 am, or with prior notification	Collect swabs in either Brain Heart Infusion broth or a commercial transport medium, such as Remel M4. Up to 5 swabs per 3ml tube; up to 11 swabs per 5.5ml tube of medium.
Avian Reovirus (AGID) (Viral Arthritis)	Rollins/Viro	Serum	Ice pack	Volume dependent	1-2 days	Charles River test antigen
Avian Reovirus (ELISA)	Rollins/Viro	Serum	Ice pack	Volume dependent	1-2 days	IDEXX kit
Avian Reovirus (PCR)	Rollins/Mol	Tendons, or tendon swabs	Ice pack	M	1-2 days	None
Bartonella culture	Rollins/Bacti	Ante-mortem: Blood in EDTA tube, lymph node or bone marrow aspirates. Postmortem: heart valves, lymph nodes.	Ice pack	M-F	21-30 days	None
Blood culture	Rollins/Bacti	Blood collected in blood culture medium	None	M-F	7-10 days	Ship unrefrigerated by express mail. Clotted blood or blood collected in EDTA are NOT suitable for culture.
Bluetongue Virus (cELISA)	Rollins/Viro	Serum	Ice pack	As needed	1 day	VMRD kit
Bovine Coronavirus (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None28	M-F	2-3 days	None

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Bovine Leukosis Virus (ELISA)	Rollins/Viro	Serum	Ice pack	F	1 day	VMRD kit
Bovine Respiratory Syncytial Virus (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
Bovine Viral Diarrhea Virus (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	3 days	Fixed ear notch samples from multiple animals should be submitted in separate containers with the animal's identification clearly marked on the container.
Bovine Viral Diarrhea Virus Antigen Capture (ELISA)	Rollins/Viro	Serum, fresh ear notch	Ice pack	W	1-2 days	Ear notch samples in individual labeled tubes without fixatives. Serum samples from precolostral newborn calves or calves older than 3 months of age. IDEXX kit
Bovine Viral Diarrhea Virus (PCR)	Rollins/Mol	EDTA whole blood, serum, nasopharyngeal swab, tissues, or ear notch	Ice pack	W	Same day if received before 10:00 am or with prior notification	None
Bovine Viral Diarrhea Virus Type 1a (VN)	Rollins/Viro	Serum	Ice pack	F	3-7 days	NVSL viral strain NADL Singer type 1a
Bovine Viral Diarrhea Virus Type 2 (VN)	Rollins/Viro	Serum	Ice pack	F	3-4 days	NVSL strain 125 genotype 2

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Brachyspira spp. culture	Rollins/Bacti	Ante-mortem: fecal or rectal swabs in anaerobic transport medium. Post-mortem: affected colon with contents, feces.	Ice pack	M-F	10-14 days	None
Brucella abortus (BAPA)	Rollins/Sero Western	Serum	Ice pack	M-F	1-2 days	Completed USDA-APHIS VS Form 4-33 (Brucellosis Test Record) must accompany bovine and swine samples if testing is for regulatory purposes.
Brucella abortus (Card)	Rollins/Sero	Serum	Ice pack	M-F	1-2 days	Equine, cervidae, BAPA positive samples
Brucella spp. culture	Rollins/Bacti	Ante mortem: milk, vaginal discharges, semen, blood cultures, lymph node aspirates. Post-mortem: lymph nodes, reproductive organs, mammary tissue, spleen, aborted fetus with membranes, placenta	Ice pack (except blood cultures)	M-F	14-16 days	For brucellosis "reactor" animals, specimens must be received with identification tag.

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Bulk Tank Milk culture	Rollins/Bacti	5-10 mls of milk collected in a sterile, leakproof container.	Ice pack or frozen	M-F	3-5 days for aerobic 14-21 days for Mycoplasma	Provides detailed information on the specific types of bacteria in the bulk tank sample and is used to screen herds for the presence/ absence of contagious mastitis pathogens, including Mycoplasma. Please notify lab if submitting more than 10 samples at one time.
C-Kit	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	Tumor/cell marker for mast cells and gastrointestinal stromal tumors can provide prognostic information in canine mast cell tumors.
Calculi Identification	Rollins/Tox	Stones, clean and dry	Ice pack	M-F	3-5 days	Compositional analysis (%) of biologically generated stones.
Campylobacter reproductive (*Vibrio*) culture	Rollins/Bacti	Preputial scrapings or semen; cervical or vaginal mucus in appropriate transport medium. Postmortem aborted fetus.	Ice pack	M-F	5-10 days	Contact lab prior to submittal for special sample collection and transport instructions.
Campylobacter intestinal	Rollins/Bacti	Rectal/fecal swabs; fresh diarrheic feces., tied-off loops of intestine.	Ice pack	M-F	3-7 days	Swabs should be collected in Cary-Blair or other transport medium suitable for maintaining camplyobacters. Must be received within 24-48 hours of collection.

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Canine Coronavirus (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
Canine Distemper (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
Canine Influenza (PCR)	Rollins/PCR	Fresh lung tissue, nasal swabs or trachea swabs	Ice pack	Tues, W, and F	Same day if received by 10:30 am, or with prior notification	Collect swabs in either Brain Heart Infusion (BHI) broth or a commercial transport medium, such as Remel M4. Up to 5 swabs per 3ml tube; up to 11 swabs per 5.5ml tube of medium.
Canine Parvovirus (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
Caprine Arthritis Encephalitis (cELISA)	Rollins/Viro	Serum	Ice pack	Thur	1-2 days	VMRD kit
CD20 (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	Tumor/cell marker for identification of B cells

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
CD3 (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	Tumor/cell marker for identification of T-cells
Chicken Anemia Virus 1:10 (CAV) (ELISA)	Rollins/Viro	Serum	Ice pack	Volume dependent	1-2 days	IDEXX kit
Clostridium chauvoei (FAT)	Rollins/Bacti Western	Affected tissues	Ice pack	W (Rollins) M-F (Western)	Rollins - batched & performed once a week. 1-2 days	Used to detect "Blackleg".
Clostridium difficile toxin assay	Rollins/Bacti	Fresh diarrheic feces or colon contents, 3-5 gm	Ice pack or frozen	F	Same day (If samples received before noon)	Detects toxins A and B. Validated for swine and equine samples
Clostridium novyi (FAT)	Rollins/Bacti	Affected tissues	Ice pack	Thur (Rollins)	Batched & performed once a week. 1-2 days	None
Clostridium perfringens culture	Rollins/Bacti	Affected sections of intestinal tract	Ice pack	M-F	3-5 days	None
Clostridium perfringens genotyping (PCR)	Rollins/Mol	Bacterial isolate	Ice pack	Thur	1-2 days	None
Clostridium septicum (FAT)	Rollins/Bacti	Affected tissues	Ice pack	Thur	Batched & performed once a week. 1-2 days	None
Clostridium sordellii (FAT)	Rollins/Bacti	Affected tissues	Ice pack	Thur	Batched & performed once a week. 1-2 days	None
Coccidia (AO)	Rollins/Viro	Feces	None	Volume dependent	1-2 days	None
Coxiella burnetii (Q Fever)	Rollins/Sero	Serum	Ice pack	As needed	3-5 days	None

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Creelan Cal-Mex RNA (PCR)	Rollins/Mol	Tracheal or oropharyngeal swabs, up to five per pool	Ice pack	Tues, W, F	Same day if received by 10:30 am, or with prior notification	Collect swabs in either Brain Heart Infusion broth or a commercial transport medium, such as Remel M4. Only done if APMV-1 matrix is positive.
Cryptosporidium (AO)	Rollins/Viro	Feces	None	Volume dependent	1-2 days	None
Cryptosporidium FA	Rollins/Bacti Western	Feces or reptilian stomach contents, fresh or preserved in 10% formalin or SAF	Ice pack	M (Rollins) M-F (Western)	Rollins - batched & performed once a week. 1-2 days	Fresh feces and reptilian stomach content must be received within 24 hours of collection. Immunofluorescence assay that detects oocysts.
Cytology	Rollins/Cyto	Aspirated samples on microscope slides or effusions/fluids in EDTA tube	Ice pack for fluids	M-F	1 day	Protect from formalin or formalin fixed tissue
Darkfield exam	Rollins/Bacti	Tissues, body fluids, feces	Ice pack	M-F	1 -2 days	Used to detect the presence of spirochetes in clinical materials.
Diff-Quick stain (hematologic)	Rollins/Bacti	Smears on microscope slides, tissues, body fluids	Ice pack	M-F	1-2 days	Used to detect the presence of fungal hyphae or *Dermatophilus congolensis* in cytologic specimens.
E. coli genotyping (PCR)	Rollins/Mol	Bacterial isolate	Ice pack	W	1-2 days	Contact lab for virulence factors that are available.
Eastern Equine Encephalitis Virus (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Equine Herpes Virus 1 (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
Equine Herpes Virus 1 (PCR)	Rollins/Mol	Whole blood (EDTA), tissues, nasopharyngeal swabs	Ice pack	As needed - Volume dependent	2 days	The test differentiates between neuropathogenic and nonneuropathogenic/ respiratory strains of EHV-1.
Equine Herpes Virus 4 (PCR)	Rollins/Mol	Whole blood (EDTA), tissues, nasopharyngeal swabs	Ice pack	As needed - Volume dependent	2 days	None
Equine Infectious Anemia (AGID)	Rollins/Viro Western	Serum	Ice pack	M-F	1-2 days	3 days for Friday testing-report Monday. There is an additional charge for priority/rush testing, call the Laboratory for availability. Samples received after 3pm will be tested the next day. VMRD kit.
Equine Infectious Anemia (ELISA)	Rollins/Viro Western	Serum	Ice pack	M-F	1 day	Same day service VMRD kit. There is an additional charge for priority/rush testing, call the Laboratory for availability.
Equine Rhinopneumonitis (VN)	Rollins/Viro	Serum	Ice pack	Tues	3-7 days	None

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Fecal Examination - centrifugation/ sucrose	Rollins/Bacti Northwestern Western	At least 5 grams of fresh feces	Ice pack	M-Thur	1-2 days	Quantitative analysis Fresh feces must be received within 24 hours of collection. Recommended for detecting camelid infections with *Eimeria macusaniensis*.
Fecal flotation	Rollins/Bacti Griffin Arden Elkins	At least 5 grams of fresh feces	Ice pack32	M-Fri	1-2 days	Quantitative analysis Fresh feces must be received within 24 hours of collection. Recommended for detecting camelid infections with *Eimeria macusaniensis*.
Fecal Examination- Modified McMaster's	Rollins/Bacti Griffin Northwestern Western	At least 5 grams of fesh feces of cattle, sheep, goats, camelids, or horses.	Ice pack	M-F	1-2 days	Quantitative analysis. Fresh feces must be received within 24 hours of collection. Please notify lab if submitting more than 10 samples at one time.
Feline Coronavirus (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
Feline Herpes Virus I (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Feline Leukemia Virus (FeLV) (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
Feline Panleukopenia (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
Ferret Coronavirus (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
Fungal Culture	Rollins/Bacti	Affected tissues, hairs, skin scrapings, scabs	Ice pack (exceptions: zygomycosis and pythiosis suspects)	M-F	14-30 days	Swab specimens are not recommended. Submit specimens for dermatophyte culture in a clean, paper envelope.
Giardia FA	Rollins/Bacti Western	Feces or reptilian stomach contents, fresh or preserved in 10% formalin of SAF.	Ice pack	M (Rollins) M-F (Western)	1-2 days	Fresh feces and reptilian stomach contents must be received within 24 hours of collection. Immunofluorescence assay that detects cysts.
Gram stain	Rollins/Bacti	Tissues, aspirates, exudates, or impression smears.	Ice pack	M-F	1-2 days	Used in the direct examination of specimens for the presence/ absence of bacteria, especially in normal sterile body fluids, abscess fluids and specimens from soft-tissue infections.

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Gross Exam (Necropsy)	Rollins/Path Griffin Northwestern Western	Freshly dead animal		M-F (Weekend and Holiday: call Laboratory on call vet)	Preliminary oral report 1-2 days Final report 7-14 days	Necropsy examination includes additional testing such as histopathology, bacteriology, and virology as necessary to make a diagnosis.
Hemagglutination Test	Rollins/Viro	Viral Isolate	Ice pack	As needed	1 day	Typically used for hemagglutinating viral isolates obtained by chicken embryo isolation
Histopathology	Rollins/Histo	Formalin-fixed tissue	None	M-F	2 days	Samples requiring special stains, additional fixation or decalcification may have an increased turnaround time
Histopathology Recuts	Rollins/Histo	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	Includes H & E sections, unstained sections on + slides and thick cut fixed tissue curls for PCR
IBA 1 (IHC)	Rollins/IHC	Formalin-fixed tissue	None	M-F	2-3 days	Tumor/cell marker for identification of histiocytic cells
Infectious Bovine Rhinotracheitis Virus (VN)	Rollins/Viro	Serum	Ice pack	Tues	3-7 days	NVSL viral strain CO
Infectious Bronchitis (PCR)	Rollins/Mol	Tracheal or oropharyngeal swabs, up to five per pool	Ice pack	F	1 day	None
Infectious Bronchitis Virus - Ark (HI)	Rollins/Viro	Serum	Ice pack	Volume dependent	2-3 days	Charles River test antigen
Infectious Bronchitis Virus - Conn (HI)	Rollins/Viro	Serum	Ice pack	Volume dependent	2-3 days	Charles River test antigen
Infectious Bronchitis Virus - Delaware (HI)	Rollins/Viro	Serum	Ice pack	Volume dependent	2-3 days	Charles River test antigen

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Infectious Bronchitis Virus - Mass (HI)	Rollins/Viro	Serum	Ice pack	Volume dependent	2-3 days	Charles River test antigen
Infectious Bronchitis Virus (ELISA)	Rollins/Viro	Serum	Ice pack	Volume dependent	1-2 days	IDEXX kit
Infectious Bronchitis Virus JMK (HI)	Rollins/Viro	Serum	Ice pack	Volume dependent	2-3 days	Charles River test antigen
Infectious Bursal Disease (AGID)	Rollins/Viro	Serum	Ice pack	M-Thur	1-2 days	Charles River test antigen
Infectious Bursal Disease (ELISA)	Rollins/Viro	Serum	Ice pack	Volume dependent	1-2 days	IDEXX kit
Infectious Laryngotracheitis Virus (PCR)	Rollins/Mol	Tracheal or oropharyngeal swabs, up to three per pool	Ice pack	Tues, W, F	Same day if received by 10:30 am, or with prior notification	Collect swabs in Remel M4 transport medium
Influenza Antigen Capture (ELISA)	Rollins/Viro	Tracheal swab pool	Ice pack	M-F	1 day	Up to 5 swabs per pool collected in 3ml of Brain Heart Infusion (BHI) Broth
Influenza Virus (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	Type A influenza strains including H7N7, H7N3, H7N2, H3N8, H3N2, H1N1, and H1N2
Lawsonia intracellularis (PCR)	Rollins/Mol	Fresh, affected intestinal segments or feces.	Ice pack	M	1-2 days	Because the organism is shed intermittently in the feces, multiple samples may be necessary to detect its presence.
Leptospira autumnalis (MAT)	Rollins/Sero	Serum, fetal fluid	Ice pack	Thurs	1-7 days	None
Leptospira bratislava (MAT)	Rollins/Sero	Serum, fetal thoracic fluid	Ice pack	Thurs	1-7 days	None
Leptospira canicola (MAT)	Rollins/Sero	Serum, fetal thoracic fluid	Ice pack	Thurs	1-7 days	None

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Leptospira grippotyphosa (MAT)	Rollins/Sero	Serum, fetal thoracic fluid	Ice pack	Thurs	1-7 days	None
Leptospira hardjo (MAT)	Rollins/Sero	Serum, fetal thoracic fluid	Ice pack	Thurs	1-7 days	None
Leptospira icterohaemorrhagiae (MAT)	Rollins/Sero	Serum, fetal thoracic fluid	Ice pack	Thurs	1-7 days	None
Leptospira MAT (5 serovars)	Rollins/Sero	Serum, fetal thoracic fluid	Ice pack	Thurs	1-7 days	Includes serovars *pomona*, *canicola*, *icterohaemorrhagiae*, *grippotyphosa*, and *hardjo*
Leptospira MAT (6 serovars)	Rollins/Sero	Serum, fetal thoracic fluid	Ice pack	Thurs	1-7 days	Includes serovars *pomona*, *canicola*, *icterohaemorrhagiae*, *grippotyphosa*, *hardjo* and *bratislava*
Leptospira pomona (MAT)	Rollins/Sero	Serum, fetal thoracic fluid	Ice pack	Thurs	1-7 days	None
Leptospira sp. (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	Leptospira *interrogans* (includes serovars *canicola*, *grippotyphosa*, *hardjo*, *icterohaemorrhagiae*, & *pomona*)
Listeria spp. culture	Rollins/Bacti	Ante-mortem: Uterine discharges, mastitic milk, cerebrospinal fluid. Postmortem: brain stem (neural form), liver (visceral form), placenta, fetus (abortion). Silage.	Ice pack	M-F	3-4days	None

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Listeria (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
Melan A (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	Tumor/cell marker expressed in melanocytes. Used for identification of poorly differentiated melanomas.
MUM-1	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	Tumor/cell marker for identification of plasma cells
Mycobacterium spp. culture	Rollins/Bacti	Skin lesions, draining tract swabs, biopsies, tissues with granulomatous lesions, feces, body fluids.	Ice pack	M-F	Up to 21 days	Detects rapid growing species. This method will not detect *Mycobacterium avium* subsp. *paratuberculosis* or *M. bovis*. Unable to culture M. hyopneumoniae
Mycoplasma gallisepticum (ELISA)	Rollins/Sero Griffin Northwestern	Serum	Ice pack	Volume dependent	3-5 days	Zoetis kit
Mycoplasma gallisepticum (HI)	Rollins/Sero	Serum	Ice pack	M-F	3-5 days	Confirmatory test for plate or ELISA positive samples
Mycoplasma gallisepticum (plate)	Rollins/Sero Northwestern	Serum	Ice pack	M-F	1-2 days	Screening test

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Mycoplasma gallisepticum (PCR)	Rollins/Mol	Tracheal swabs, up to three per pool	Ice pack	Tues, W, F	Same day if received by 10:30 am, or with prior notification	Collect swabs in a mycoplasmal transport medium.
Mycoplasma hyopneumoniae (ELISA)	Rollins/Sero	Serum	Ice pack	As needed	2-3 days	IDEXX kit
Mycoplasma meleagridis (ELISA)	Rollins/Sero Griffin	Serum	Ice pack	Volume dependent	3-5 days	Zoetis kit
Mycoplasma meleagridis (HI)	Rollins/Sero	Serum	Ice pack	M-F	3-5 days	Confirmatory test for ELISA positive samples
Mycoplasma spp. culture	Rollins/Bacti	Ante-mortem: Tracheal exudates and aspirates, eggs, milk, choanal or conjunctival or inner ear swabs, joint fluid, vaginal swabs. Post-mortem: lung tissue with bronchi, trachea, sinuses, air sacs, intact affected joint.	Ice pack	M-F	Up to 21 days	Swabs in suitable transport medium, such as Amie's. Delivered within 48 hours of collection. Frozen tissue specimens are suitable. Inhouse speciation of isolates for which there is an additional cost is limited to *Mycoplasma gallisepticum* and *M. synoviae*.
Mycoplasma synoviae (ELISA)	Rollins/Sero Griffin Northwestern	Serum	Ice pack	M-F	3-5 days	Zoetis kit
Mycoplasma synoviae (HI)	Rollins/Sero	Serum	Ice pack	M-F	3-5 days	Confirmatory test for plate or ELISA positive samples
Mycoplasma synoviae (plate)	Rollins/Sero Northwestern	Serum	Ice pack	M-F	1-2 days	Screening test
Mycoplasma synoviae (PCR)	Rollins/Mol	Tracheal swabs, up to three per pool	Ice pack	Tues, W, F	Same day if received by 10:30 am, or with prior notification	Collect swabs in a mycoplasma transport medium.

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
NAHLN influenza matrix - (PCR)	Rollins/Mol	Single nasal swabs or fresh lung tissue	Ice pack	M, W	Same day if received by 10:30 am, or with prior notification	Pooled nasal swabs are not acceptable. Test only performed upon request.
Neospora caninum (ELISA)	Rollins/Sero	Serum	Ice pack	As needed	2-3 days	Used to detect antibodies in serum of cattle, sheep, and goats. IDEXX kit
Neospora caninum (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
NPIP *Salmonella* culture (conventional)	Rollins/Bacti Griffin	Drag swabs, chick papers, foot covers, litter, fluff, cloacal swabs placed in double - strength skim milk. Live reactor birds.	Ice pack	M-Thur	7-10 days	Serogrouping is included. For serotyping, isolates are forwarded to the National Veterinary Services Laboratory (NVSL).
Pancytokeratin AE1/AE3 (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	AE1/AE3—Tumor/cell marker used for identification of epithelial cells
Parainfluenza 3 (VN)	Rollins/Viro	Serum	Ice pack	Tues	3-7 days	NVSL viral strain SF-4
PAX-5 (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	Tumor/cell marker for identification of B-cells

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
PNL2 (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	Tumor/cell marker expressed in melanocytes. Used for identification of poorly differentiated melanomas.
Porcine Circovirus 2 (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
Porcine Coronavirus - TGE (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
Porcine Coronavirus-TGE (PCR)	Rollins/Mol	Intestinal swab or fresh diarrheic feces	Ice pack	Thur	1 day	For same day results, notify lab and submit specimens by 10am.
Porcine delta-corona Virus (PCR)	Rollins/Mol	Intestinal swab, fresh diarrheic feces, fresh intestine, oral fluids, environmental swabs	Ice pack	M, W, F	1 day	Call lab for submission of large numbers of samples or expedited testing.
Porcine Epidemic Diarrhea Virus (PCR)	Rollins/Mol	Intestinal swab, fresh diarrheic feces, fresh intestine, oral fluids, environmental swabs	Ice pack	M, W, F	1 day	Call lab for submission of large numbers of samples or expedited testing.
Porcine Parvovirus (HI)	Rollins/Viro	Serum	Ice pack	Volume dependent	2-3 days	NVSL test antigen

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Porcine Reproductive & Respiratory Syndrome Virus (ELISA)	Rollins/Viro	Serum	Ice pack	As needed	1-2 days	IDEXX kit
Porcine Reproductive & Respiratory Syndrome Virus (PCR)	Rollins/Mol	Fresh lung tissue, fetal thymus, fetal thoracic fluid, tonsil, serum, or lung lavage fluid	Ice pack	M, Thur	1-2 days	None
Porcine Reproductive & Respiratory Syndrome Virus (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
Pseudorabies Virus g1 (ELISA)	Rollins/Viro	Serum	Ice pack	M, W, F	1 day	Submit sera with NC Swine Test Chart IDEXX kit
Pseudorabies Virus gB (ELISA)	Rollins/Viro	Serum	Ice pack	M, W, F	1 day	Submit sera with NC Swine Test Chart IDEXX kit

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Rabies Testing (not performed by NCVDLS)	Rollins/Nec Griffin Northwestern Western	Freshly dead animal, head.		M-F	1 day	The NCVDLS is not to be utilized for the shipping, case management, and client notification of companion animal and wildlife/feral rabies suspects involving human exposure. Local and county animal control or Public Health agencies and private veterinarians are advised to directly submit specimens for testing to the State Lab of Public Health in Raleigh. NCVDLS will process and submit livestock and equine samples where local personnel do not have the expertise or equipment to safely procure a sample. An organ pluck fee will be charged for brain removal if necropsy is not performed.
Ruminant Mastitis culture	Rollins/Bacti	5-10 ml of milk collected in a sterile, leak-proof container.	Ice pack or frozen	M-F	3-5 days	Please notify lab if submitting more than 10 samples at one time.
Salmonella pullorum (tube)	Griffin	Serum	Ice pack	M, Tues	3 days	Requirement for NPIP

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Salmonella spp. culture	Rollins/Bacti	Ante-mortem: Fecal swabs or 10-15 gm of feces from diarrheic animals. A minimum of 3 specimens collected on consecutive days is preferred. Post-mortem: Intestines, liver, gall bladder, spleen, lung, lymph nodes, bone marrow, feces, and intestinal contents.	Ice pack	M-F	3-5 days	Animal must have a minimum of five consecutive *Salmonella* negative cultures before being considered not to have salmonellosis. **Note: Group D Salmonella spp. isolates, from poultry, will be serotyped.**
Swine Influenza Virus H1N1 (HI)	Rollins/Viro	Serum	Ice pack	Volume dependent	2-3 days	A/SW/Iowa/73 strain
Swine Influenza Virus H3N2 (HI)	Rollins/Viro	Serum	Ice pack	Volume dependent	2-3 days	A/SW/NC/35922/98 strain
Swine Influenza Virus (PCR) (Nucleoprotein)	Rollins/Mol	Fresh lung, bronchial or nasal swabs (up to five per pool), or lung lavage fluid	Ice pack	M, W	1-2 days	Test is not confirmatory for pH1N1 influenza strains. See NAHLN influenza matrix and NAHLN N1 for pH1N1 strain detection.
Swine Influenza Virus subtyping (PCR)	Rollins/Mol	Fresh lung, bronchial or nasal swabs, or lung lavage fluid	Ice pack	M, W	1-2 days	Test is not confirmatory for pH1N1 influenza strains
Toxicology Evaluation	Rollins/Tox	Rumen/reticulum content, stomach content (EQ, SA), suspect bait, hay, forage, water	Ice pack	M-F	3-5 days	May detect toxic plant, blue-green algae, blister beetle, battery plate, pesticide bait forms; may indicate further testing.

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Toxic Plant Identification	Rollins/Tox	Suspected plant		M-F	3-5 days	Entire plant, if possible (flowers and fruits help). Non-toxic plants may not be identified.
Toxoplasma gondii (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
Tritrichomonas foetus exam	Rollins/Bacti	Feline feces/intestinal contents; bovine cervical mucus or preputial scrapings	None	M-F	6-12 days	Contact lab prior to submittal for special sample collection and transport instructions. Samples must be submitted in InPouch medium.
Turkey coronavirus (PCR)	Rollins/Mol	Fresh intestine cloacal swab	Ice pack	M, Thur	2 days	None
Vimentin (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	Tumor/cell marker for mesenchymal cells
Virus Isolation (cell culture)	Rollins/Viro	Fresh affected tissue	Ice pack	Tues, Thur	2-4 weeks	Turnaround time is from day of inoculation for specimen, bacterial contamination will require longer passage period for results
Virus isolation (egg inoculation)	Rollins/Viro	Fresh affected tissue	Ice pack	Tues, F	2-3 weeks	Infectious Bronchitis Virus requires 6 passages with 3-week turnaround time

AGENT/ PROCEDURE	LAB/ SECTION	SPECIMEN(S)	COOLANT	TEST DAYS	TURNAROUND TIME (WORKING DAYS)	COMMENTS
Von Willebrand Factor (VWF) (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	Tumor/cell marker for endothelial cells
West Nile Virus (IHC)	Rollins/IHC	Formalin-fixed tissue or paraffin-embedded tissue on charged microscope slides	None	M-F	2-3 days	None
West Nile Virus (PCR)	Rollins/Mol	Brain stem, cerebellum, and cerebrum	Ice pack	Tues	2 days	None
Yersinia spp. culture	Rollins/Bacti	Diarrheic feces, visceral organs, or lymph nodes with lesions	Ice pack	M-F	3-21 days	None

VI. Reportable Diseases

In the state of North Carolina, veterinarians are required by law (G.S. 106-307.2) to report the following diseases to the State Veterinarian's Office at (919)707-3250.

- Anthrax
- Avian Chlamydiosis (Psittacosis, ornithosis)
- Avian Encephalomyelitis
- Avian Influenza (High Pathogenic)
- Avian Influenza (Low Pathogenic)
- Brucellosis (livestock only)
- Classical Swine Fever (Hog Cholera)
- African Swine Fever
- Foot and Mouth Disease
- Contagious Equine Metritis
- Echinococcosis
- Equine Encephalitis
    - Eastern Equine Encephalitis
    - Venezuelan Equine Encephalitis

    - Western Equine Encephalitis
    - St. Louis Encephalitis
- Equine Infectious Anemia
- Exotic Newcastle Disease
- Foreign Animal Diseases (including, in addition to those listed in this Rule, any disease believed to be absent from the United States and its territories)
- Fowl Typhoid (*Salmonella gallinarum*)
- Infectious Laryngotracheitis (other than vaccine induced)
- Leishmaniasis
- *Mycoplasma gallisepticum/Mycoplasma synoviae*
- Paramyxovirus (other than Newcastle; includes menangle virus)
- Plague (*Yersinia pestis*)
- Pseudorabies
- Pullorum (*Salmonella pullorum*)
- Q fever (*Coxiella burnetii*)
- Rabies (equine and livestock only)
- Scabies (cattle and sheep only)
- Screw Worm (Exotic myiasis)
- Transmissible Spongiform Encephalopathies (including Bovine Spongiform Encephalopathy, Chronic Wasting Disease, and Scrapie)
- Tuberculosis
- Tularemia (*Francisella tularensis*)
- Vesicular Disease
    - Foot and Mouth
    - Vesicular Stomatitis
    - Vesicular Exanthema
    - Swine Vesicular Disease
- West Nile (domestic animals only)

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