

North Carolina Veterinary Diagnostic Laboratory System

User Guide



Established in 1950 by the
North Carolina Department of Agriculture

Guide prepared by:

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Revised 2009

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NCVDLS Website address:

www.ncvdl.com

I. Introduction

The North Carolina Veterinary Diagnostic Laboratory System (NCVDLS) was established in 1950 by the North Carolina Department of Agriculture to assist owners and their veterinarians in diagnosing diseases of livestock and poultry, and to prevent outbreaks of catastrophic foreign animal diseases by early detection. Regulatory testing is also performed by the diagnostic laboratories when required for exporting, importing, and interstate movement of livestock and poultry. The number of laboratory accessions has steadily increased through the years and now includes a large number of companion animal submissions.

Rollins Laboratory in Raleigh is the central laboratory, with branch laboratory locations in Arden, Elkin, Monroe, and Rose Hill. The laboratories are open Monday through Friday, 8 am to 5 pm and accept all animal species for examination. Emergency after hours, weekend, and holiday submissions must be arranged by contacting the laboratory.

Rollins Animal Disease Diagnostic Laboratory

State Veterinarian: Dr. David Marshall
Director of Laboratories: Dr. Karen Post
Head of Microbiology: Dr. Gene Erickson

Mammalian Pathologists: Dr. Pete Moisan, Dr. Steve Rushton, Dr. Alison Tucker
Avian Pathologist: Dr. Tahseen Aziz
Veterinary Diagnosticians: Dr. Jennifer Haugland, Dr. Stacy Robinson, Dr. Mahogany Wade

FedEx/UPS: 2101 Blue Ridge Road
Raleigh, NC 27607
US Mail: 1031 Mail Service Center
Raleigh, NC 27699-1031
Phone: (919) 733-3986
Fax: (919) 733-0454

Western Animal Disease Diagnostic Laboratory

Director: Dr. Richard Oliver
Veterinary Diagnostician: Dr. David Drum
FedEx/UPS: 785 Airport Road
Fletcher, NC 28732
US Mail: PO Box 279
Arden, NC 28704
Phone: (828) 684-8188

Northwestern animal Disease Diagnostic Laboratory

Director: Dr. Darrell Rector, Jr.
Veterinary Diagnostician: Dr. Kim Townsend
FedEx/UPS/US Mail:
1689 N. Bridge Street
Elkin, NC 28621
Phone: (336) 526-2499
Fax: (336) 526-2603

Hoyle C. Griffin Animal Disease Diagnostic Laboratory

Director: Dr. Kim Hagans
Veterinary Diagnostician: Dr. Reginald Ridenhour
FedEx/UPS: 401 Quarry Road
Monroe, NC 28112
US Mail: PO Box 2183
Monroe, NC 28111
Phone: (704) 289-6448
Fax: (704) 283-9660

Rose Hill Animal Disease Diagnostic Laboratory

Director: Dr. Carlton Rouse
Veterinary Diagnostician: Dr. Timothy McComb
FedEx/UPS: 329 Yellow Cut Road
Rose Hill, NC 28458
US Mail: PO Box 37
Rose Hill, NC 28458
Phone: (910) 289-2635
Fax: (910) 289-2070

II. NCVDL Current Fee Schedule

No out of state samples will be tested at any of the laboratories in the NCVDL System.

The following is a list of fee-based test services. For necropsies, disposal fees are based on weight of the carcass: \$5.00 (0-100 lbs.); \$15.00 (101-500 lbs.); \$30.00 (over 500 lbs.) . Serologic fees are on an individual sample basis.

Companion Animal Services (includes gross exam and ancillary diagnostic assays)

Small Companion Animal Necropsy	\$30.00 (includes disposal fee)
Large Companion Animal Necropsy	\$25.00 (Horses, Llama, etc)
Disposal Fee*	\$5.00, \$15.00, \$30.00

Food Animal Services (includes gross exam and ancillary diagnostic assays)

Food Animal Necropsy	No Charge
Disposal Fee	\$5.00, \$15.00, \$30.00
Organ Pluck (Tissue Handling Fee)	\$5.00 (assessed when large fresh tissue specimens are sampled and disposed)

Histopathologic Services

Companion Animal	
Cytology	\$10.00
Mail-in Necropsy Fixed Tissue	\$30.00
Surgical Biopsies	\$30.00

Microbiologic Culturing Services

Companion Animal Microbiology \$10.00

Viral Serologic Services

Equine Infectious Anemia \$6.00

Serologic Processing and Handling

Serum Separation \$1.00 per sample (Not to exceed \$5.00 per accession)

Specimen Referral Shipping and Handling

Fee varies from \$2.50 to \$25.00 based on package weight and destination.

National Poultry Improvement Plan Service

Please consult website for pricing on National Poultry Improvement Plan Services. <http://www.ncvdl.com/VetLabFees.html>

Test Results

Online test results are available in real-time for registered veterinary clinics and corporate clients. Instructions on how to register are on the website. <http://www.ncvdl.com/VetLabServicesUserAccountSetup.htm>

III. Submitting Specimens

Submission Forms:

All specimens must be accompanied by a completed submission form. Submission forms can be found on the NCVDL website <http://www.ncvdl.com> or you can request packets of forms by calling Rollins Animal Disease Diagnostic Laboratory at (919) 733-3986. There are three different forms to be used for the following submissions:

Avian/Poultry: for all avian samples including tissues, live/dead birds, biopsy, and cytology samples. *Exception: specimens for avian influenza, M. gallisepticum, M. synoviae, M. meleagridis must also be accompanied by the appropriate USDA forms.*

Surgical Biopsy and Cytology Submission Form – for all cytology samples (fluid or slides) and biopsy samples obtained from live animals. Bacterial or fungal culture of these samples can also be requested using this form.

General Submission Form – for all other types of samples, including: serum, tissues, swabs, necropsy specimens.

Fill out the forms completely including owner information and animal identification. Please list samples submitted and make a specific test request in the appropriate blank. The User's Guide will help to identify which tests are offered within NCV DLS. For some agents (e.g. BVDV) there are different tests offered. If forms are incomplete or if specific tests are not requested, sample processing/testing will be delayed.

Fresh tissues submitted for laboratory testing should be packed individually and sealed in leak-proof containers or plastic bags, surrounded by ice packs and placed in an insulated box to slow decomposition. It is especially important to package intestines separately to prevent contamination of other tissues. Refer to Bacteriology Laboratory Section for additional guidelines regarding collecting and submitting specimens for bacterial/fungal isolation.

Blood samples deteriorate quickly—please package them so they will stay cool during transit. Serum samples should be poured off the clot and sent either cooled or frozen. If serum is not spun down, a serological processing and handling fee of \$1.00 per sample (not to exceed \$5.00 per accession) will be assessed. If paired samples are to be tested, the acute serum should be held frozen and sent to the laboratory with the convalescent serum.

Any contaminated, decomposed, or inappropriate specimens will not be processed.

IV. Pathology Services

The Pathology Section offers necropsy services and histopathological evaluation of tissue samples. Telephone consultation with a pathologist or diagnostician prior to submission may be useful and is particularly encouraged if a case is complex or the diagnostic investigation is an ongoing series of submissions.

Necropsy Services Intact carcasses should be submitted as soon as possible after death. Live animals are preferred only in the following situations: porcine neonatal diarrhea, poultry, fish. Refusal of an animal for necropsy at any time will be 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).

Necropsies are performed at each laboratory from 8 am to 5 pm, Monday through Friday. Animals received late in the day may not be necropsied until the following day. As of July 12, 2005 necropsies will not be performed on State holidays or after 5 pm on weekdays, unless they qualify as an emergency. At the Rollins Laboratory facility only, routine weekend necropsies will be limited to 8 am to 12 pm on Saturdays. No routine necropsy services are available on Saturdays 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' laboratory veterinarian is available after hours by telephone for consultation purposes. Lay Clients with non-emergency cases will be encouraged to have their animals necropsied by their veterinarian to prevent a decomposed animal from being presented to the laboratory on the following business day. Likewise, veterinary clients will be encouraged to perform their own necropsies during after hours and holidays. See Field Necropsy Guidelines below.

Remains of animals submitted for necropsy can not be released from the laboratory, per state statute, unless prior arrangements have been made by the client for cremation by a commercial crematory service. Requests for individual cremation must be clearly written on the submission form.

Preliminary results of the necropsy examination will be reported by telephone to the client. Often, additional laboratory procedures are required to arrive at a diagnosis. A final written report will be mailed to the client.

Legal/Cruelty Cases In accordance with our mission statement, animal cruelty/legal cases will be handled in the same manner as a routine necropsy submission. Additional veterinary forensic testing such as determining the time of death, forensic entomology, or determining types of accelerants used in burn cases will not be performed. Please notify the laboratory at the time of submission that the case may involve cruelty or a legal issue. Any pictures of the animal must be taken prior to submission of the animal to the diagnostic laboratory, as pictures will not be taken during the gross necropsy. We are currently unable to perform toxin testing at our laboratory. For suspected poisoning cases, samples may be outsourced for toxicology testing, at the client's request and with the client being responsible for the cost. When poisoning is suspected, please notify the laboratory at the time of submission. When bullet retrieval is desired in suspected gunshot cases, radiographs should be taken and brought to the laboratory upon submission of the animal for necropsy.

Rabies testing is performed on fresh brain tissue by the North Carolina State Laboratory of Public Health located in Raleigh. The NCVDL is not to be utilized for the transshipping, case management, and client notification of companion animal and wildlife/feral rabies suspects involving human exposure. Please review the Rabies Sample Submittal Policy on pages 26-27.

If only rabies testing is needed, fresh brain tissue or the head of small companion animals or wildlife should be submitted directly to the State Laboratory in a leak-proof insulated container with ice packs via FedEx, UPS, courier, or county animal control officer and include a completed DHHS Form #1614.

State Laboratory of Public Health
306 N. Wilmington Street
Raleigh, NC 27611
(919) 733-7544 (7:30 am-4:30 pm weekdays)
(919) 310-5620 (4:30 pm Friday – 12 pm Saturday)
(919) 707-5900 State Public Health Veterinarian
<http://slph.state.nc.us/virologyserology/rabies/default.asp>

If diagnostic tests, including those for rabies are requested, please ship specimens to the closest NCVDLs facility and the brain tissue will be forwarded to the Rabies Laboratory after processing for the additional test requests is completed. Include a completed NCVDLs Submission Form and a completed DHHS Form #1614 with each submission.

Field Necropsy Guidelines Submission of tissue specimen (s) is often the best method of obtaining a diagnosis. However, proper selection and preservation of samples is essential to make the most efficient and economical use of the laboratory. The three conditions that most frequently interfere with diagnosis are (1) post mortem autolysis, (2) sample collection too late in the course of disease, and (3) inappropriate sample selection. When collecting tissues from necropsied animals, the history, clinical signs, and gross lesions should determine which tissues are collected.

Please call the appropriate laboratory and talk to one of the veterinary pathologists or diagnostician at any time if you have questions concerning specimen selection or preservation.

1. Specimens for histopathology should include multiple slices of the appropriate organs, including the lesion, 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 1/8 ” in the critical first 24 hours of fixation) and placed in a leak-proof, wide-mouthed solid container with 10 times the volume of formalin to tissue.
2. Fresh specimens should be large enough to demonstrate the lesion yet small enough to allow for rapid chilling. Ideally, fresh samples should be packaged individually to prevent cross-contamination and properly labeled. It is absolutely vital NOT to package fresh intestine with other tissues, as this results in fecal contamination of other organs. See Bacteriology and Molecular Diagnostic sections for further information about sample selection and preservation.

Histopathology/Cytology Services A complete history including animal age, breed, and sex is required for histopathology. The location, size, and duration of masses is required for biopsy/cytology specimens. Include information about diagnostic tests and treatments performed and indicate the method of obtaining the sample (i.e. excisional biopsy, punch biopsy, aspirate of mass, aspirate of cavity fluids, etc) so that interpretations of findings will be more accurate and comments by the pathologist can be more in depth. Tumors should be placed in a leak-proof, wide-mouthed solid container with 10 times the volume of formalin to tissue. Do not incise into the tumors. Tissues fixed for at least 2 days can be shipped in smaller volumes of formalin.

All the following listed services are available at the Rollins Laboratory facility; branch laboratories offer limited services. Please refer to Section XII, Index of Laboratory Tests by Species, pages 25-28 for availability.

V. Bacterial Serology Laboratory Section

The time required for completing tests is approximate and does not include holidays and weekends. Results may be delayed if tests must be repeated; if confirmation by another procedure is needed; or if a sudden increase in the caseload occurs. Always collect and submit samples well in advance of your deadline to provide us with ample time should problems arise.

BOVINE	SAMPLE	METHOD	TIME	WHEN TESTED
**Anaplasmosis	Serum 1 ml	CF & ELISA	3-7 days	Friday
**Brucellosis	Serum 5 ml	Several tests	3-7 days	Daily
Brucellosis	Milk 5ml	BRT	3-4 days	Daily
(Paired samples preferred)				
PORCINE				
**Brucellosis	Serum 5 ml	Several tests	1 week	Daily
<i>Mycoplasma hyopneumoniae</i>	Serum 2 ml	ELISA	1 day	Once per week
EQUINE				
Brucellosis	Serum 1 ml	Several tests	1 week	Daily
POULTRY				
<i>*Mycoplasma gallisepticum</i>	Serum 0.5 ml	ELISA	3-4 days	As needed
	Serum 0.5 ml	HI	3-4 days	As needed
	Serum 0.5 ml	Plate AGG	3-4 days	As needed
<i>*Mycoplasma synoviae</i>	Serum 0.5 ml	ELISA	3-4 days	As needed
	Serum 0.5 ml	HI	3-4 days	As needed
	Serum 0.5 ml	Plate AGG	3-4 days	As needed
<i>*Mycoplasma meleagridis</i>	Serum 0.5 ml	ELISA	1 day	As needed
	Serum 0.5 ml	HI	3-4 days	As needed
	Serum 0.5 ml	Plate AGG	3-4 days	As needed
<i>*Salmonella pullorum/typhoid</i>	Serum 0.5 ml	Tube AGG	3 days	As needed
<i>Bordetella avium</i>	Serum 0.5 ml	ELISA	Contact	As needed
Laboratory				
CANINE				
Brucellosis	Serum 5 ml	Referral to NVSL		
OVINE/CAPRINE				
**Brucellosis	Serum 5 ml	Several tests	3-7 days	Daily
ALL SPECIES				
<i>Leptospira</i> sp.	Serum 1 ml	MA	5-10 days	Twice weekly

VI. VIROLOGY LABORATORY SECTION

Viral Serology Section

<u>BOVINE</u>	<u>SAMPLE</u>	<u>METHOD</u>	<u>TIME</u>	<u>WHEN TESTED</u>
Bovine Leukosis	Serum 2 ml	ELISA	2 days	As needed
Bluetongue	Serum 2 ml	AGID=REFERRAL		
	Serum 2 ml	cELISA	2 days	Once weekly as needed
Johne's disease	Serum 2 ml	ELISA, AGID, Referral	2 days	Once weekly as needed
Enzootic abortion (Chlamydial)	Serum 2 ml	CF	Referral	
<i>Neospora caninum</i> (limit 14/herd)	Serum 2 ml	ELISA	2 days	Once weekly as needed
PI3	Serum 1 ml	SN	7 days	Once weekly as needed
IBR (paired samples preferred)	Serum 1 ml	SN	5 days	Once weekly as needed
BVD-Types I & II	Serum 2 ml	SN	5 days	Once weekly as needed
 <u>PORCINE</u>				
/Pseudorabies	Serum 2 ml	G1 Screen	3 days	Mon.-Fri
	Serum 2 ml	LATEX AGG	1 day	Mon.-Fri
TGE/Respiratory Coronavirus	Serum 2 ml	Referral	1-2 weeks	As arranged
Parvovirus (Paired samples preferred)	Serum 2 ml	HI	5 days	Once weekly as needed
PRRS	Serum 2 ml	ELISA	3 days	Twice weekly
HEV	Serum 2 ml	Referral		
Swine Influenza (H1N1, H3N2)	Serum 2 ml	HI	3 days	Once a week
 <u>EQUINE</u>				
/EIA	Serum 2 ml	AGID and ELISA	2 days	Daily
Rhinopneumonitis (EHV-1) (Paired samples preferred)	Serum 2 ml	SN	5 days	Once weekly as needed
 <u>POULTRY</u>				
Infectious bronchitis				
Mass.	Serum 2 ml	HI	2 days	Once weekly as needed
Ark.	Serum 2 ml	HI	2 days	Once weekly as needed
JMK	Serum 2 ml	HI	2 days	Once weekly as needed
Conn.	Serum 2 ml	HI	2 days	Once weekly as needed
Del 072	Serum 2 ml	HI	2 days	Once weekly as needed
Avian Encephalomyelitis	Serum 2ml	AGID	1-2 days	As needed
Avian influenza	Serum 0.5 ml	AGID, ELISA	1-2 days	As needed
Avian influenza (chicken only)	Serum .05 ml	ELISA	1 day	Twice weekly as needed
Viral arthritis	Serum 0.5 ml	AGID	1-2 days	As needed
Turkey hemorrhagic enteritis	Serum 0.5 ml	AGID	1-2 days	As needed
Adenovirus-127	Serum 0.5 ml	HI	1 day	As needed
Infectious bursal disease (Gumboro)	Serum 0.5 ml	SN	1 day	As needed
APMV1	Serum 0.5 ml	HI	1 day	W, Th, F
<i>Chlamydophila psittaci</i>	Serum 0.5 ml	Referral		

OVINE

OPP	Serum 2 ml	AGID	2-5 days	As needed
Enzootic abortion (chlamydial)	Serum 2 ml	CF	Referral	
	Fetal tissues	Agn Capture	2 days	As needed
Johne's Disease	Serum 2 ml	AGID	2 days	Once weekly
Johne's Disease	Serum 2 ml	ELISA	2 days	Once weekly

CAPRINE

CAE	Serum 1 ml	cELISA	2 days	As needed
CAE	Serum 2ml	AGID	2-5 days	As needed
Johne's Disease	Serum 2 ml	AGID	2 days	Once weekly as needed
Johne's Disease	Serum 2ml	ELISA	2 days	Once weekly as needed
Enzootic abortion (chlamydial)	Serum 2ml	CF	Referral	
	Fetal tissues	Agn Capture	2 days	As needed

CERVINE

EHD	Serum 2 ml	AGID	2 days	Weekly as needed
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Virology Section

BOVINE

IBR	Lung, trachea, spleen	FATST	1 day	Daily
	Lung, trachea, spleen, nasal swab	FACCT	2-4 weeks	Tues & Thurs
BVD	Spleen, blood, ileum, colon	FACCT	2-4 weeks	Tues & Thurs
Coronavirus	Intestine, feces (10 ml)	EM	3 days	Weekly as needed
Rotavirus	Intestine, feces (10 ml)	LATEX AGG	1 day	Daily
	Intestine, feces (10 ml)	EM	3 days	Weekly as needed
Poxvirus, Papilloma	Skin lesion	EM	3 days	Weekly as needed
BRSV	Lung	ELISA	1 day	Daily
<i>Chlamydophila psittaci</i>	Fetal tissue, fluids, and	ELISA	1 day	Daily

PORCINE

Pseudorabies	Brain, tonsil	FATST	1 day	As needed
	Brain, tonsil	FACCT	48 hours	M,W,F
TGE	Small intestine	FATST	1 day	Daily
PRRS	Serum	FACCT	2 weeks	As arranged
	Tissues, Lung, LN	FATST	1 day	As needed
Rotavirus	Intestinal contents	LATEX AGG	1 day	Daily
	Intestinal contents	EM	3 days	Weekly as needed
Parvovirus	Lung (mum. fetus)	FATST	1 day	Daily
Respiratory Coronavirus	Pneumonic lung	FATST	1 day	As needed
Swine Influenza	Lung	FACCT	2 weeks	As needed
Swine Pox	Skin lesion	EM	3 days	Weekly as needed
<i>Mycoplasma hyopneumoniae</i>	Lung	FATST	1 day	As needed
Virus isolation	Tissues	FACCT PSK 15, ST	2 weeks	As needed

EQUINE

Rhinopneumonitis (EHV-1)	Lung	FATST	1 day	As needed
	Lung	FACCT	2-4 weeks	As needed
EIA	Spleen	AGID	2 days	As needed
Influenza	Lung, nasal swab	ELISA	1 day	As needed

CANINE

Coronavirus	Intestine	FATST	1 day	As needed
	Lung, tonsil, urinary bladder, brain,			
Paramyxovirus (Distemper)	conjunctival swabs	FATST	1 day	As needed

FELINE

Feline Leukemia	Tears, saliva, blood, tissues, bone marrow	Referral		
Feline Immunodeficiency Virus (FIV)	Tears, saliva, blood, tissues, bone marrow	Referral		
Feline Rhinotracheitis	Lung	FATST	1 day	As needed
Feline pneumonitis (Chlamydiosis)	Lung	ELISA	1 day	As needed

POULTRY

APMV1	Lung, trachea, brain	CE	2 weeks	Thursday
Infectious Bronchitis (IBV)	Lung, trachea	CE	2 weeks	Thursday
Infectious Laryngotracheitis	Trachea	CE	3 days	Mon. & Tues.
Encephalomyelitis	Brain	CE	2 weeks	Thursday
Avian influenza	Lung, trachea	CE	2 weeks	Thursday
	Tracheal swab	ELISA	1 day	As needed
Fowl pox	Lesion	CE	3 days	Mon. & Tues.
Infectious Bursal Disease (IBD)	Bursa of Fabricius	AGID	2 days	As needed
Viral Arthritis	Tendons & tendon sheaths	CE	2 weeks	Thursday
Ornithosis (<i>Chlamydothyla psittaci</i>)	Choanal swab or swab of fresh liver, spleen or lesions	ELISA	1 day	As needed
	Feces/transport medium	FACCT	1 week	As needed
AE embryo susceptibility	2 dozen fertile eggs	VN	3 weeks	Thursday

ALL SPECIES

<i>Cryptosporidium</i>	Feces	Auramine-O	1 day	Tues & Thurs
<i>Coccidia</i>	Feces	Auramine-O	1 day	Tues & Thurs
Enteric and Dermatopathic Viral Diseases	Tissues, fluids, feces	EM	3 days	Weekly as needed

VIROLOGICAL AND SEROLOGICAL TEST PROCEDURES

AGG- Agglutination	FACCT- Fluorescent Antibody Cell Culture Technique
AGID- Agar Gel Immunodiffusion	FATST- Fluorescent Antibody Tissue Section Technique
Agn-Antigen Capture	HI- Hemagglutination-Inhibition
BRT-Brucellosis Ring Test	IFA- Indirect Fluorescent Antibody
CE-Chicken Embryo	MA- Microagglutination
CF-Complement Fixation	PCR- Polymerase Chain Reaction
ELISA-Enzyme Labeled Immunosorbent Assay	SN- Serum Neutralization
EM-Electron Microscopy	VN- Virus Neutralization

For all serological test procedures:

*Charges are assessed for these procedures

**Must be submitted on official forms signed by an accredited veterinarian.

VII. Immunohistochemistry Services

Immunohistochemistry is defined as the detection of antigens in tissue sections by means of an immunologic reaction. This service is currently available on a limited basis as an ancillary diagnostic test to support histopathologic diagnoses on necropsy cases.

Animal Species	AntigenTest	Formalin Fixed Specimen
Porcine	Circovirus	Lung, tonsil, ileum, lymph node, spleen, liver
	Porcine respiratory coronavirus	Ileum; lymph node
	Porcine respiratory and reproductive virus	Lung, tonsil; lymph node
	Swine influenza virus	Lung
	Transmissible gastroenteritis virus	Ileum; jejunum
Bovine	Bovine coronavirus	Spiral colon; ileum
	Bovine viral diarrhea virus	Ileum, thymus; spleen; mucosal lesions, skin (ear notch)
	<i>Neospora caninum</i>	Brain; heart; skeletal muscle
Avian	Infectious laryngotracheitis virus	Affected sections of upper trachea; eyelids
Canine	Parvovirus	Affected sections of intestine; heart
	Coronavirus	Affected sections of intestine; heart
Feline	Panleukopenia	Intestine
	Coronavirus	Lung, brain, kidney, lymph node
Multiple species	West Nile Virus	Brain; spinal cord (equine) Brain, heart, spleen, liver, kidney (avian)

VIII. Molecular Diagnostics Laboratory Section

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 sensitive method used to specifically 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 are essential to ensure reliable test results. Nucleic acid integrity must be maintained throughout these processes.

Please note: Most tests require prior consultation with laboratory personnel before they will be performed. These are denoted by an asterisk.

General requirements

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. Materials in other types of swabs can be inhibitory to PCR.

Testing Services

****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 a commercial medium formulated for *Mycoplasma* transport (Remel MicroTest M4 Transport Media). 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 three swabs may be placed in tubes of transport medium. Tests are performed routinely on Tuesday and Friday or on an as needed basis with results being available in 24 hours.

***E. coli* genotyping**

Disease associated with *E. coli* infections relies on the differentiation of pathogenic from non-pathogenic strains. To cause disease, enterotoxigenic *E. coli* must produce 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.

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. Tests are run on Wednesdays with results being available an average of 48 hours later.

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. Tests are performed on an as needed basis with results being available in 24 hours.

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

***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 broth or a commercial transport medium (Remel MicroTest M4 Transport Media, up to five swabs may be pooled into 3 ml tube of medium). Dry swabs are unsuitable for testing. Samples should be shipped with cold packs for next day delivery. Testing is on an as needed basis with results available in 24 hours.

***Exotic Newcastle Disease**

Exotic Newcastle disease (END), previously known as velogenic viscerotropic Newcastle disease, is a highly contagious and fatal viral disease affecting all avian species. END is so virulent that many birds die without showing any clinical signs (severe respiratory, central nervous, or digestive disturbances).

Tracheal or oropharyngeal swabs must be submitted in Brain Heart Infusion broth or Remel MicroTest M4 Transport Medium. Up to five swabs may be pooled into 3 ml tube of medium. Dry swabs are unsuitable for testing. Samples should be shipped with cold packs for next day delivery. Testing is on an as needed basis with results available in 24 hours.

Porcine Reproductive and Respiratory Syndrome

PRRS virus was first observed in the US in the mid 1980's and in Europe in 1990. The syndrome was initially called Mystery Swine Disease since no previously known pathogens could be implicated. This widespread disease is associated with epidemic abortions, infertility, and acute pneumonia. Our PCR assay is able to discriminate between US and European virus.

Please indicate if screening for only one type of virus (US or European), which requires different reagents.

Fresh lung tissue, fetal thymus, fetal thoracic fluid, serum, 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. Tests are performed each Tuesday and results are available in 24-36 hours.

Please note: At this time, the laboratory does not provide routine herd monitoring services for PRRS.

***Transmissible gastroenteritis**

TGE is a rapidly-spreading viral illness of swine of all ages. The disease, which is characterized by vomiting and profuse diarrhea, results in severe dehydration and high mortality in piglets. PCR is best suited to a Dacron swab of the very fluid intestinal contents found in the small intestine beginning at the level of the jejunum. Fresh diarrheic material collected on the farm is also suitable for testing. Place specimens in a sealed tube, such as a red top serum collection tube, to prevent desiccation. Samples should be refrigerated after collection and shipped to the lab with cold packs for next day delivery. For same day results, specimens should be received by 10:00 AM. As PCR is a very expensive test, it will be reserved only for suspected clinical outbreaks.

Swine influenza

Swine influenza virus is an acute, highly contagious respiratory disease. Lung tissue, bronchial or nasal swabs, or lung lavage fluid should be submitted for testing. Samples should be stored overnight in the refrigerator and mailed with cold packs for next day delivery. Testing is performed on an as needed basis with results available in 24-36 hours.

West Nile/Eastern Equine Encephalitis

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. Eastern equine encephalitis, commonly referred to as EEE, is a viral disease of wild birds that is transmitted to horses and humans also by mosquitoes. The virus attacks the central nervous system causing erratic behavior and lack of coordination. For both viral PCR tests, brain stem, cerebellum, and cerebrum from clinically-affected horses should be submitted for testing. These sections should be placed in individual bags, labelled, and stored in the refrigerator until they can be shipped to the lab with cold packs delivery. Testing is performed on an as needed basis with results available in 24-36 hours.

Bovine viral diarrhea virus (BVDV)

BVD is the agent of bovine mucosal disease which primarily causes reproductive disorders and economic loss in cattle. The assay is performed on serum. BVDV is also a cause of abortions and illness in camelids; blood collected in EDTA must be submitted. Limit of 50 animals per herd.

IX. Bacteriology Laboratory Section

The Bacteriology Laboratory 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 on large animal or avian specimens, isolation and identification of *Tritrichomonas foetus*, 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 of specimens.
3. Avoid swab specimens, if possible. Swabs are easily contaminated and most often do not provide a sufficient volume of specimen for culture.
4. Place specimens in an appropriate transport device to maintain a buffered and non-nutritive environment and to prevent desiccation. Specimens submitted in expired transport devices will not be processed.
5. Place multiple tissue specimens in separate containers to prevent cross-contamination.
6. Use an indelible marker to label specimens with the location (tissue) and animal species of origin.
7. Generally, maintain specimens at refrigeration temperature immediately after collection and send to the laboratory with cold packs.
8. Package specimens carefully to ensure there will be no leakage or breakage in transit.
9. Always indicate the test (s) request,
10. Different microbiologic culture procedures require different times for completion:
 - ◆ Aerobic, routine (culture/sensitivity) 3-4 days
 - ◆ Anaerobic 7-10 days
 - ◆ *Brucella* species 14 days
 - ◆ *Campylobacter* 3-7 days
 - ◆ Fungal 2-4 weeks
 - ◆ *Listeria monocytogenes* cold enrichment 12 weeks
 - ◆ *Mycobacterium avium* ssp. *paratuberculosis* (Johnes ' s) 16 weeks
 - ◆ *Mycoplasma* species 3 weeks

Please refer to the chart on the following pages for submission guidelines based upon suspected disease.

CONDITION	POSSIBLE ETIOLOGIC AGENTS	SPECIMEN (S)	COLLECTION/ TRANSPORT
Abortion	<i>Brucella</i> species <i>Campylobacter</i> species <i>Leptospira</i> species <i>Listeria monocytogenes</i> 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	<i>Aspergillus</i> spp. zygomycetes	Placenta with cotyledons. Fetal stomach contents and/or skin lesions.	Refrigerated.
Abscess	<i>Actinomyces</i> spp. Anaerobes <i>Arcanobacterium pyogenes</i> <i>Pasteurella multocida</i> staphylococci streptococci	Exudate or swab in transport medium. Biopsy in sterile saline.	Room temperature.
Actinomycosis or actinobacillosis	<i>Actinomyces bovis</i> <i>Actinobacillus lignieresii</i>	Exudate with granules or lesion. Swab of abscess material in transport medium or collected in syringe w/o needle.	Room temperature.
Anaerobic infections	Clostridia <i>Bacteroides</i> spp. <i>Fusobacterium</i> spp. <i>Prevotella</i> spp. <i>Porphyromonas</i> spp.	Large piece of affected tissue. Exudates collected in anaerobic transport medium. Ligated segments of affected intestine.	Room temperature.
Anthrax	<i>Bacillus anthracis</i>	Blood sample taken from superficial vein, such as ear. Necropsy is contraindicated. Spleen or lymph nodes, if necropsy has been performed.	Refrigerated. Must state on submission form that case may involve an anthrax suspect.
Arthritis	<i>Streptococcus suis</i> <i>Arcanobacterium pyogenes</i> <i>Haemophilus</i> species <i>Erysipelothrix rhusiopathiae</i> <i>Mycoplasma</i> species <i>Staphylococcus aureus</i>	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.	Room temperature.
Atrophic rhinitis of swine	<i>Bordetella bronchiseptica</i> and/or <i>Pasteurella multocida</i>	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	<i>Bartonella</i> 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	<i>Clostridium chauvoei, novyi, perfringens, septicum, sordelli</i>	Fresh piece of muscle with lesion. Impression smear slides from affected tissue for fluorescent antibody test.	Room temperature.
Botulism	<i>Clostridium botulinum</i>	Food suspected of containing toxin. Ligated sections of fresh intestine. Large section of liver. Serum. Samples may be forwarded to NVSL.	Refrigerated tissues. Frozen serum.
Bovine respiratory disease	<i>Histophilus somni</i> <i>Pasteurella multocida</i> <i>Mannheimia haemolytica</i> <i>Mycoplasma</i> 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	<i>Brucella</i> 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.
Campylobacteriosis (bovine and ovine)	<i>Campylobacter fetus</i> ss. <i>venerealis</i> , or ss. <i>fetus</i> <i>Campylobacter jejuni</i>	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 24-48 hours of collection. Frozen tissue specimens are also acceptable.
Campylobacteriosis (canine and equine)	<i>Campylobacter jejuni</i>	Fresh rectal/fecal swabs, fresh diarrhetic feces collected in Cary-Blair or other medium suitable for maintaining <i>Campylobacterium</i> viability.	Refrigerated. Delivered within 24-48 hours of collection. Frozen specimens also acceptable.

Caseous lymphadenitis	<i>Corynebacterium pseudotuberculosis</i>	Affected lymph node; abscesses, exudates in sterile container; swabs in aerobic transport medium.	Refrigerated.
Colibacillosis	<i>Escherichia coli</i>	Affected portions of intestines; fresh feces or fecal swabs in aerobic transport medium.	Refrigerated.
Cystitis	<i>Escherichia coli</i> <i>Proteus</i> species <i>Enterococcus</i> species <i>Staphylococcus aureus</i>	5-10 ml of fresh urine in sterile container. Bladder swabs in aerobic transport medium.	Refrigerated.
Dermatophytosis (ringworm)	<i>Microsporum</i> and <i>Trichophyton</i> species	Skin scrapings or hairs. Swabs unsuitable.	Room temperature.
Dermatophilosis (“ rain rot ”)	<i>Dermatophilus congolensis</i>	Scabs and crusts in sterile container. Skin biopsy in sterile saline.	Room temperature.
Enterotoxemia	<i>Clostridium perfringens</i>	Several ounces of fresh intestinal contents in sterile container.	Refrigerated. Frozen specimen preferable.
Enteritis (diarrhea)	<i>Escherichia coli</i> <i>Salmonella enterica</i> Others	Fresh, diarrheic feces in sterile container. Tied-off loops of affected intestine.	Refrigerated.
Erysipelas	<i>Erysipelothrix rhusiopathiae</i>	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	<i>Staphylococcus hyicus</i> <i>Streptococcus</i> species	Skin scrapings in a sterile container. Skin swabs in aerobic transport medium.	Refrigerated.
Glasser's disease (“ HPS ”)	<i>Haemophilus parasuis</i>	Brain, heart, lung, and intact, swollen joints or other organs with fibrinous coating. Swabs are not acceptable.	Refrigerated.
Johne's disease	<i>Mycobacterium avium</i> ss. <i>paratuberculosis</i>	Ante-mortem: Fecal samples in sterile container. Post-mortem: Ileocecal valve, mesenteric lymph nodes, mucosal scrapings.	Refrigerated. Frozen acceptable. *Samples are batched and processed once per week.
Keratoconjunctivitis, bovine	<i>Moraxella bovis</i> and <i>ovis</i>	Conjunctival swabs in aerobic transport medium.	Refrigerated. Must arrive at lab within 24 hours of collection.
Interdigital dermatitis “ Footrot ”	<i>Dichelobacter nodosus</i> <i>Fusobacterium necrophorum</i>	Surgical biopsy of affected tissue in anaerobic transport medium	Room temperature

Leptospirosis	<i>Leptospira</i> species	Ante-mortem: urine in a sterile container or special <i>Leptospira</i> transport medium (if available). Post-mortem: kidney, liver, ocular fluid, fetal brain.	Refrigerated. Urine for darkfield examination must be collected in an equal volume of 10% formalin.
Listeriosis	<i>Listeria monocytogenes</i>	Neural form: brain stem. Visceral form: liver. Abortion form:- placenta and fetus or fetal stomach contents. Feed samples.	Refrigerated.
Mastitis	<i>Staphylococcus</i> species <i>Streptococcus</i> species <i>Mycoplasma</i> 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	<i>Streptococcus</i> species <i>Streptococcus suis</i> <i>Histophilus somni</i> (<i>Haemophilus somnus</i>) <i>Cryptococcus neoformans</i>	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 <i>Mycobacterium</i> spp.	Skin lesions, draining tract swabs, biopsies, tissues with granulomatous lesions, feces, body fluids.	Refrigerated.
Mycoplasmosis	<i>Mycoplasma</i> 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	<i>Nocardia asteroides</i> and other species	Biopsy in sterile container. Aspirates, exudates (to include granules). Transtracheal wash in sterile container.	Room temperature.
Otitis externa	<i>Proteus</i> species <i>Pseudomonas</i> species <i>Staphylococcus</i> species <i>Streptococcus</i> species Many other bacteria Yeasts (<i>Malessezia</i>)	Ear swab placed in aerobic transport medium. Impression smear slide.	Refrigerated. Room temperature.
Pleuropneumonia of swine (" APP ")	<i>Actinobacillus pleuropneumoniae</i>	Portion of affected lung or other tissues with lesions.	Refrigerated.

Proliferative enteritis	<i>Lawsonia intracellularis</i>	Affected portion of ileum or fresh feces.	Refrigerated.
Pseudomembranous colitis	<i>Clostridium difficile</i>	Affected portion of colon; colon contents in anaerobic transport medium.	Refrigerated/frozen.
Pyelonephritis, bovine	<i>Corynebacterium renale</i>	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	<i>Rhodococcus (Corynebacterium) equi</i>	Ante-mortem: Transtracheal wash in sterile container. Post-mortem: Fresh lung with lesions and respiratory lymph nodes.	Refrigerated.
Salmonellosis	<i>Salmonella enterica</i>	Ante-mortem: Fecal swabs or 1-5 gm of feces from diarrheic animals. A minimum of 3 specimens collected on 3 sequential days are 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. Post-mortem: Heart blood, bone marrow, spleen, liver, lungs.	Room temperature. Refrigerated.
Sporotrichosis	<i>Sporothrix schenckii</i>	Biopsy material from unopened skin nodules or scrapings from skin ulcers, placed in sterile containers with saline.	Refrigerated.
Strangles, equine	<i>Streptococcus equi</i> ss. <i>equi</i>	Abscess material on swab in aerobic transport medium or in syringe w/o needle.	Refrigerated.
Swine dysentery/ spirochetal colitis	<i>Brachyspira hyodysenteriae</i> <i>Brachyspira pilosicoli</i> 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)	<i>Blastomyces dermatitidis</i> <i>Coccidioides immitis</i> and <i>posadasii</i> <i>Histoplasma capsulatum</i> <i>Cryptococcus neoformans</i>	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	<i>Mycobacterium</i> 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.
Tularemia	<i>Francisella tularensis</i>	Heart blood, liver, spleen, bone marrow, or other organs with white necrotic foci.	Refrigerated. Must state on submission form tularemia suspect.

Parasitology Submission Guidelines

Proper collection and submission of samples to the laboratory is essential for the accurate diagnosis of parasitic infection. Parasitology services are limited to the following procedures. Please note that these services are not provided by all laboratories within the NCVDL system.

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 and disintegrated. Nematode eggs often hatch rendering them more difficult to identify. Also, free-living soil nematodes, 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.

Fecal flotation, direct examination services and McMaster's quantitation are available at all laboratories within the NCVDLs. Specimens should be submitted in individual sealed containers; plastic specimen cups, Whirl-pak® 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.

Parasite Identification

Parasite identification is performed at NCSU/CVM through a cooperative agreement. Specimens should be sent to the Rollins Laboratory. Helminth 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.

Trichostrongylus axei 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 trichostrongylids in felines. This medium is 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 minimum of 7 days for results.

Cryptosporidium and *Giardia*

The Rollins Laboratory offers a direct immunofluorescent assay for the detection of cryptosporidiosis and giardiasis. The test is performed once weekly on Mondays with results available the same day. Specimens should be submitted in 10% formalin (1:1 ratio). Specimens should **not** be preserved in polyvinyl alcohol.

Please note that the following services are not available: small animal fecal flotations, heartworm checks, and Baermann exams.

X. Reportable Diseases

If any of the following diseases or any foreign animal disease is suspected, pertinent information should be immediately reported by telephone to the State Veterinarian 's Office at (919) 733-7601. If Psittacosis is suspected in a pet bird, please contact the Veterinary Public Health Office at (919) 733-3410.

02 NCAC 52C .0603 REPORTABLE DISEASES

All persons practicing veterinary medicine in North Carolina shall report the following diseases and conditions to the State Veterinarian 's office by telephone within two hours after the disease is reasonably suspected to exist:

Anthrax;
Avian Chlamydiosis (Psittacosis, ornithosis);
Avian Encephalomyelitis;
Avian Influenza (High Pathogenic);
Avian Influenza (Low Pathogenic);
Brucellosis (livestock only);
Classical Swine Fever (Hog Cholera);
Contagious Equine Metritis;
Echinococcosis;
Equine Encephalomyelitis (including Eastern Equine Encephalomyelitis, Venezuelan Equine Encephalomyelitis, Western Equine Encephalomyelitis, and St. Louis Encephalomyelitis);
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 Scrapies);
Tuberculosis;
Tularemia (*Francisella tularensis*);
Vesicular Disease (Foot and Mouth, vesicular Stomatitis, Vesicular Exanthema, Swine Vesicular Disease);
West Nile (domestic animals only).

XI. Policy Statements

**North Carolina Department of Agriculture & Consumer Services
Veterinary Division
Rollins Animal Disease Diagnostic Laboratory**

July 12, 2005

MEMORANDUM

TO: Rollins Laboratory Veterinarians

FROM: Drs. David Marshall and Karen Post

SUBJECT: Necropsy Policies

To improve our laboratory system, we are adopting changes in our necropsy policy. Effective August 1, 2005, the following policies will be implemented for all necropsy submissions:

1. Necropsies will not be performed on State holidays or after 5 pm on weekdays, unless they qualify as an emergency. Weekend necropsies will be limited to 8 am-12 pm on Saturdays. Emergency situations are limited to (a) cases of multiple death loss within a herd/flock over a short period of time (24-48 hours) in which case the animals are not autolytic (b) cases of suspected foreign animal disease (foot and mouth, exotic Newcastle, etc.) (c) zoonotic diseases. An “ on-call ” laboratory veterinarian will be available by telephone during these times for consultation purposes. Lay clients who have animals that are not considered as “ emergency ” cases should be encouraged to have their animals necropsied by their veterinarian to prevent a decomposed animal from being presented to the laboratory for necropsy at a later date. Likewise, veterinary clients should be encouraged to perform their own necropsies.
2. Refusal of an animal for necropsy at any time will be at the discretion of the veterinarian assigned to the case 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 for an animal has already been obtained and confirmed, therefore a necropsy is unwarranted. e.g. fractured leg, uterine prolapse, or chronic laminitis.
3. If the necropsy diagnosis is obvious, such as gunshot wound or gastric torsion, no tissues should be submitted for histological diagnosis.

NORTH CAROLINA DEPARTMENT OF AGRICULTURE
AND CONSUMER SERVICES
Veterinary Division- Diagnostic Laboratory System
Raleigh, North Carolina

NCVDLS DIVISION NOTICE	1-05	9-15-05
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Rabies Sample Submittal Policy

Purpose: The purpose of this notice is to outline procedures for the receipt, handling, and submittal to the State Laboratory of Public Health (SLPH) of rabies suspect specimens by the NCDA&CS Veterinary Diagnostic Laboratory facilities.

Cancellation: none

References:

- 1) NCVDLS Rabies specimen submittal guidelines <http://www.ncvdl.com>
- 2) NCDHHS SLPH Form 1614
- 3) NCDHHS Rabies specimen collection/submittal guidelines <http://slph.state.nc.us/>
- 4) NCDHHS Instructions for the Submission of Animal Heads and Bats for Rabies Testing

Procedures:

- 1) As rabies is a zoonotic disease with potentially serious public health implications, the SLPH is the designated testing facility for all post-mortem human, companion animal, livestock, and wildlife rabies suspect samples. The NCVDLS is not approved for nor has the capability of rabies testing and, therefore, refers all specimen samples to the SLPH for analysis.
- 2) As NCVDLS veterinarians are not formally trained public health practicing medical personnel, the veterinary diagnostic laboratory system is not to be utilized for the transshipping, 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 to the SLPH, packaging the specimens according to the Federal Shipping requirements (49 CFR 170) for diagnostic specimens. The SLPH currently accepts whole heads of cats, dogs, and other small mammals properly chilled and shipped with the appropriate paperwork.
- 3) The NCVDLS will process and submit livestock and equine samples where local personnel do not have the expertise or equipment to safely procure a sample. The Case Coordinating Veterinarian will contact the Local Health Department in the county of residence of the owner and alert them to the submittal. A Local Health Department representative will be listed as the contact person in Box #7 of DHHS Form 1614 in addition to the NCVDLS veterinarian. An organ pluck fee of \$5.00 will be charged for those large animals where brain removal is necessary.

- 4) Walk-in citizen submissions to the NCVDLS for rabies testing are discouraged. Clients will be encouraged to coordinate with their local Health Department or animal control agency for submittal and testing where possible. Cases will be handled on a case by case basis. NCVDLS veterinarians will not turn away requests involving human or animal exposure. In those instances where the NCVDLS does submit the sample, no additional diagnostic tests will be conducted and no fee will be charged. Contact and notification protocol will be as in #3 above.

- 5) Animals of all species presented for a diagnostic work-up where rabies could be suspected in a differential diagnosis will also be conducted at NCVDLS facilities. The veterinary laboratory diagnostician assigned to the case or the appropriate county health department contact will be listed as the contact person in Box #7 of DHHS Form 1614 and be responsible for notifying the client of test results. In positive cases, the diagnostician will also notify the appropriate Health Department for follow up consultation. Documentation of telephone communications will be as per internal NCVDLS Quality Assurance SOP 's. Normal fees for diagnostic specimen necropsy and disposal will apply, depending on species.

- 6) All questions regarding human exposure, rabies vaccination, etc. should be directed to the DHHS Public Health Veterinarians at (919) 707-5900 during normal business hours (M-F, 8am-5pm) . Consultation services are available at other times at (919) 733-3419. Emergency testing over a weekend or a holiday is available if the situation meets SLPH testing criteria for weekend or holiday testing. The rabies testing personnel can be reached by calling the pager at (919) 310-5620.

Dr. David Marshall
 Director-NCVDLS

Dr. Karen Post
 Asst. Director – NCVDLS

<p>DISTRIBUTION: NCVDLS laboratory personnel, NC SLPH, County animal control agencies, County Health Departments, NCVMA</p>	<p>SUBJECT CATEGORY: Rabies sample submittal</p>
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XII. INDEX OF LABORATORY TESTS BY SPECIES

1. AVIAN TESTS

Agent/Disease	Test Method	Laboratory
Adenovirus	HI	Rollins, Virology Section
Avian influenza	AGID, ELISA, CE PCR	Rollins, Virology Section; Monroe, Rose Hill, and Elkin Lab Rollins, Molecular Diagnostics Section
Avian paramyxovirus 1	HI, CE	Rollins, Virology Section
<i>Bordetella avium</i>	HI Culture	Rollins, Serology Section; Rose Hill Lab; Monroe Lab Rollins, Bacteriology Section
Encephalomyelitis	CE, SN, ELISA	Rollins, Virology Section
<i>Chlamydia</i> (ornithosis)	ELISA, FACCT	Rollins, Virology Section
Exotic Newcastle Disease	PCR	Rollins, Molecular Diagnostics Section
Fowl pox	CE	Rollins, Virology Section
Infectious bronchitis	HI, CE	Rollins, Virology Section
Infectious bursal disease (Gumboro)	SN, CE AGID	Rollins, Virology Section; Rollins, Virology Section; Rose Hill Lab
Infectious laryngotracheitis	CE PCR IHC	Rollins, Virology Section Rollins, Molecular Diagnostics Section Rollins, Immunohistochemistry Section
<i>Mycoplasma gallisepticum</i>	Culture ELISA, HI, Plate AGG PCR	Rollins, Bacteriology Section Rollins, Serology Section; Rose Hill Lab, Monroe Lab, Elkin Lab Rollins, Molecular Diagnostics Section
<i>Mycoplasma meleagridis</i>	Culture ELISA, HI	Rollins, Bacteriology Section Rose Hill Lab
<i>Mycoplasma synoviae</i>	Culture ELISA, HI, Plate AGG PCR	Rollins, Bacteriology Section Rollins, Serology Section; Rose Hill Lab, Monroe Lab, Elkin Lab Rollins, Molecular Diagnostics Section
<i>Salmonella arizonae</i>	Tube AGG Culture	Rollins, Serology Section; Rose Hill Lab Rollins, Bacteriology Section
<i>Salmonella enteritidis</i>	Tube AGG Culture	Rollins, Serology Section; Rose Hill Lab Rollins, Bacteriology Section
<i>Salmonella pullorum/typhoid</i>	Tube AGG Culture	Rollins, Serology Section; Rose Hill Lab Rollins, Bacteriology Section
<i>Salmonella typhimurium</i>	Tube AGG Culture	Rollins, Serology Section; Rose Hill Lab Rollins, Bacteriology Section
Turkey hemorrhagic enteritis	AGID	Rollins, Virology Section
Viral arthritis	AGID, CE	Rollins, Virology Section

2. BOVINE TESTS

Agent/Disease	Test Method	Laboratory
Anaplasmosis	CF, ELISA	Rollins, Serology Section
Bluetongue	ELISA	Rollins, Virology Section
Bovine leukosis	ELISA	Rollins, Virology Section
Bovine respiratory syncytial virus	Tissue ELISA	Rollins, Virology Section
Bovine viral diarrhea	FATST, FACCT, SN PCR IHC	Rollins, Virology Section Rollins, Molecular Diagnostics Rollins, Immunohistochemistry Section
Brucellosis	Several serological assays Culture	Rollins, Serology Section Rollins, Bacteriology Section
Campylobacteriosis	Culture	Rollins, Bacteriology Section
Coccidiosis	Auramine O	Rollins, Virology Section
Coronavirus	EM IHC	Rollins, Virology Section Rollins, Immunohistochemistry Section
Cryptosporidiosis	FAT Auramine O	Rollins, Bacteriology Section Rollins, Virology Section
Enzootic abortion - chlamydial	Tissue ELISA, FACCT	Rollins, Virology Section
Giardiasis	FAT	Rollins, Bacteriology Section
Infectious bovine rhinotracheitis	SN	Rollins, Virology Section
Johne ' s disease (<i>Mycobacterium avium</i> ssp. <i>paratuberculosis</i>)	AGID, ELISA Culture	Rollins, Virology Section Rollins, Bacteriology Section
Leptospirosis	Darkfield or FA exam MAT	Rollins, Bacteriology Section Rollins, Serology Section
<i>Neospora caninum</i>	ELISA IHC	Rollins, Virology Section Rollins, Immunohistochemistry Section
Papillomavirus	EM	Rollins, Virology Section
PI3	SN	Rollins, Virology Section
Poxvirus	EM	Rollins, Virology Section
Rotavirus	EM, Latex AGG	Rollins, Virology Section
Trichomoniasis	Culture, microscopic exam	Rollins, Bacteriology Section

3. CANINE TESTS

Agent/Disease	Test Method	Laboratory
Brucellosis	Culture	Rollins, Bacteriology Section
Coccidiosis	Auramine O	Rollins, Virology Section
Coronavirus	FATST	Rollins, Virology Section
Cryptosporidiosis	FAT	Rollins, Bacteriology Section
	Auramine O	Rollins, Virology Section
Distemper virus	FATST	Rollins, Virology Section
Giardiasis	FAT	Rollins, Bacteriology Section
Leptospirosis	Darkfield or FA exam	Rollins, Bacteriology Section
	MA	Rollins, Serology Section
Parvovirus	IHC	Rollins, Immunohistochemistry Section

4. CAPRINE/OVINE TESTS

Agent/Disease	Test Method	Laboratory
Brucellosis	Several serological assays	Rollins, Serology Section
	Culture	Rollins, Bacteriology Section
CAE	cELISA, AGID	Rollins, Virology Section
Coccidiosis	Auramine O	Rollins, Virology Section
Enzootic abortion (chlamydial)	CF	Referral, NVSL
Johne 's disease (<i>Mycobacterium avium</i> ssp. <i>paratuberculosis</i>)	AGID, ELISA	Rollins, Virology Section
	Culture	Rollins, Bacteriology Section
Leptospirosis	Darkfield or FA exam	Rollins, Bacteriology Section
	MA	Rollins, Serology Section
OPP	AGID	Rollins, Virology Section
Toxoplasmosis	IFA	Rollins, Serology Section

5. EQUINE TESTS

Agent/Disease	Test Method	Laboratory
Brucellosis	Several serological assays	Rollins, Serology Section
	Culture	Rollins, Bacteriology Section
<i>Clostridium difficile</i>	Toxin ELISA	Rollins, Bacteriology Section
EIA	AGID, ELISA	Rollins, Virology Section
EEE	Tissue PCR	Rollins, Molecular Diagnostics Section
	IHC	Rollins, Immunohistochemistry Section
Influenza	ELISA, FACT	Rollins, Virology Section
Leptospirosis	Darkfield or FA exam	Rollins, Bacteriology Section
	MA	Rollins, Serology Section
Rhinopneumonitis (EHV-1)	SN, FATST, FACCT	Rollins, Virology Section
West Nile Virus	Tissue PCR	Rollins, Molecular Diagnostics Section
	IHC	Rollins, Immunohistochemistry Section

6. FELINE TESTS

Agent/Disease	Test Method	Laboratory
Coronavirus (FIP, enteric corona)	IHC	Rollins, Immunohistochemistry Section
Panleukopenia	IHC	Rollins, Immunohistochemistry Section
Pneumonitis	Tissue ELISA	Rollins, Virology Section
Rhinotracheitis	FATST	Rollins, Virology Section
Toxoplasmosis	IFA	Rollins, Serology
Trichomoniasis	Culture, microscopic examination	Rollins, Bacteriology Section

7. PORCINE TESTS

Agent/Disease	Test Method	Laboratory
Brucellosis	Several serological assays	Rollins, Serology Section
	Culture	Rollins, Bacteriology Section
Circovirus	IHC	Rollins, Immunohistochemistry Section
EMC	FACCT	Rollins, Virology Section
HEV	FACCT	Rollins, Virology Section
Influenza	ELISA, FACCT	Rollins, Virology Section
	IHC	Rollins, Immunohistochemistry Section
	PCR	Rollins, Molecular Diagnostics Section
<i>Mycoplasma hyopneumoniae</i>	ELISA	Rollins, Serology Section
	FATST	Rollins, Virology Section
Parvovirus	HI, FACCT, FATST	Rollins, Virology Section
PRRS	IHC	Rollins, Immunohistochemistry Section
	PCR	Rollins, Molecular Diagnostics Section
	ELISA, FACCT, FATST	Rollins, Virology Section
Pseudorabies	Latex AGG, FATST, FACCT	Rollins, Virology Section
<i>Lawsonia intracellularis</i>	PCR	Rollins, Molecular Diagnostics Section
Rotavirus	Latex AGG, EM	Rollins, Virology Section
Respiratory Coronavirus	IHC	Rollins, Immunohistochemistry Section
	FATST, SN	Rollins, Virology Section
Swine Pox	EM	Rollins, Virology Section
TGE	IHC	Rollins, Immunohistochemistry Section
	PCR	Rollins, Molecular Diagnostics Section
	FATST	Rollins, Virology Section