

Kord Animal Disease Diagnostic Laboratory

Laboratory Use Guide

TENNESSEE DEPARTMENT OF AGRICULTURE Division of Regulatory Services

(U.S. Postal Address)

Kord Animal Disease Laboratory
P.O. Box 40627, Melrose Station
Nashville, Tennessee 37204-0627

(Overnight Delivery Address)

Kord Animal Disease Laboratory
Ellington Agricultural Center
440 Hogan Road
Nashville, Tennessee 37220

Telephone Numbers

Information	615-837-5125
Brucellosis Results	615-837-5120
Tissue Receiving	615-837-5410
Serology	615-837-5221
Virology	615-837-5268
Bacteriology	615-837-5427
EAC Security	615-533-9945
Billing	615-837-5410
Blood/Serum boxes	615-837-5231

FAX	615-837-5250
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INTRODUCTION

The Kord Animal Disease Diagnostic Laboratory (KADDL) is the State of Tennessee's veterinary diagnostic laboratory. It is funded by legislative appropriation and fee revenue, and operates within the Tennessee Department of Agriculture, Division of Regulatory Services. No cost diagnostic services are provided to livestock and poultry producers except for a small fee charged for equine infectious anemia serology. Modest fees are also charged for companion animal diagnostic services.

The operational philosophy of the laboratory is to work through the local veterinary practitioner, who is best able to evaluate the client-producer's problems and recommend appropriate actions. The mission of the laboratory is to provide accurate and timely diagnostic services to the practitioner who serves livestock and poultry producers as well as owners of companion animals in Tennessee. We strongly recommend that case material be submitted by or at the direction of a practicing veterinarian, although owners may submit cases directly. Results of examinations, analyses, and tests will be returned to the practitioner for their consideration in formulating a diagnosis and recommending action to clients. Some test results must be reported to the State Veterinarian because of regulatory requirements (see Reportable Diseases).

If at any time there is a question regarding a test or procedure, please call ahead to be assured of that service and its submission requirements. We also appreciate your comments regarding the laboratory.

We invite veterinarians or their technicians to spend a day in the laboratory meeting the staff and observing methods used in various diagnostic procedures. This helps us understand your problems related to laboratory use and helps you in preparing submissions so that the most useful information may be obtained from a submission. We welcome you to set up an appointment for a day of laboratory observation. Time spent at the laboratory may qualify as continuing education, which is required for license renewal.

Ed McKinley, Director

Table of Contents

Hours	4
Fees	4-5
Shipping Requirements	6
Master List of Subcontractors	7
Approach to Diagnosis, Abortions	8
Bacteriology submissions	9-14
Necropsy submissions.....	15
Histopathology	16
Cytology	17-18
Parasitology	19
Serology	20-21
Equine Infectious Anemia or Coggins testing	21
BVD Ear Notch Sample Collection/Submission Requirements	22-23
Serology Tests Available and specimen requirements (by species)	24-31
Toxicology Tests Available and Samples Required	32-35
Virology, tests by species	36-38
Sources of Help in Tennessee	39-40
Reportable Diseases in Tennessee	41
Submission form, Surgical Biopsy	42
Submission form, Fresh and Fixed tissues	43
Submission form, Necropsy	44
Owner' Release from, Necropsy	45
Index	46-47

GUIDELINES OF OPERATION

A. **Laboratory hours** are 8:00 AM to 4:30 PM, Central Time. Holidays are observed for New Years, Martin Luther King's birthday, Presidents' Day, Good Friday, Memorial Day, Independence Day, Labor Day, Veteran's Day, Thanksgiving and the following day, and Christmas.

B. **Fees (subject to change, effective July 11, 2010)**

Equine Infectious Anemia \$7.00/Test

Livestock testing (used for food & fiber), Horses – No charge (\$7.00 charge for EIA/Coggins)

Companion Animals

Fixed tissue & fresh tissue (from a necropsy):

- Diagnostic fee/histopathology fee of \$35.00.(includes cytology from same site/problem).
- This fee includes histopathology and all other testing except toxicology (bacteriology, immunology, etc) if the tissue come from a necropsied animal.
- How to tell: If fresh internal organs are submitted: lung, liver, etc.

Fixed tissue & fresh tissue (from a live animal):

If fresh tissues are excised from an animal still living, i.e. skin, toe, testicle, there is a charge for each separate test: histopathology, bacteriology, fungal, serology, etc.

Pathology:

Histopathology/Biopsy	\$35.00 per animal
Necropsy (no toxicology)	70.00 per animal, limb, organ, head.
Necropsy (with toxicology)	105.00
Necropsy (with spinal cord)	95.00
Cytology	30.00 per tissue, problem
Slide preparation/Cytology	15.00 per specimen
Slide preparation/HE	8.00 per slide
Slide preparation/IHC, charged	10.00 per block
Submitter special stain	10.00 per slide, requested by submittor

Toxicology:

First test	35.00
Each additional test	15.00

Immunology:

Serum/FA, etc.	15.00 each
Lyme Disease	18.00

Virus isolation: 20.00

Bacteriology:

Aerobic culture	15.00 (per swab, tissue,site, sample)
Antibiotic sensitivity	8.00 (per isolate)
Fungal culture	15.00
Anaerobic culture	15.00
Stain, gram, acid fast	5.00

Parasitology *15.00 per test*

Miscellaneous charges:

Save Remains	50.00
Save Ashes	50.00
Disposal (animal or parts)	25.00
Shipping and Handling	20.00
Packaging fee	4.00
Sample supplies	Cost plus 10%
<u>Past due accounts</u>	<u>5.0% per month</u>

- C. All submissions should be accompanied by an appropriate submission form. The form should be filled out entirely to maximize diagnostic results.
- D. Animals should not be brought to the laboratory on an outpatient basis for collection of specimens.
- E. Reporting Results
1. A telephone or fax report will be given on any significant laboratory findings as they become available or if the result requires an explanation by a veterinary diagnostician. Please provide a legibly written telephone number, including the area code, when requesting a telephone report. Cell phone numbers help to avoid “telephone tag”.
 2. A written report will be issued to the referring veterinarian upon completion of the case unless results are transmitted via fax, in which case, the faxed copy serves as the written report.
 3. Copies of the owner’s written report are sent to the owner if the owner delivered the tissues and/or animal directly to the laboratory or the submission is from a farm animal species and the owner’s complete address is present on the submission form.
- F. Please report any changes of address, phone number or clinic association to the laboratory at this number: (615) 837-5125.
- G. Veterinarians utilizing laboratory services may choose to send payment with submitted specimens or establish a charge account with the laboratory. Accumulated charges will be billed to the clinic on a monthly basis. Accounts 90 days overdue are subject to a) withholding of fee-based services until payment is received and b) discontinuation of credit requiring payment to accompany the sample.
- H. Submitting samples, specimens, or animals to KADDL implies agreement to policies of the State of Tennessee and KADDL and transfers ownership of samples and property to KADDL upon receipt unless otherwise determine by written agreement prior to submission.

- I. Safety of Laboratory personnel is paramount. Submissions with attached needles, leaking formalin or other fluids are subject to being discarded.
- J. Animals that are submitted with the concern or suspicion of being shot and that a projectile can be recovered should realize that the laboratory does not have radiographic capabilities. Before submission, radiographs of the body should be taken (lateral and ventral-dorsal views) and submitted with the animal.
- K. Bone lesions or malformations are best diagnosed by radiographs. The laboratory does not have radiographic capabilities and has limited capacity to examine bone lesions.
- L. Formalin-fixed tissues are required for histopathology examination.

U. S. POSTAL MAILING AND PACKAGING REQUIREMENTS

for Etiologic Biological Laboratory

Specimens to the Tennessee Department of Agriculture

All etiologic and biologic specimens sent to the Department of Agriculture through the U. S. Mail must be properly packaged so the contents will not leak during shipment. Reference Domestic Mail Manual, CO23.10.0-10.7.

- The specimen must be packaged in a securely sealed watertight primary container (test tube, vial, etc.) which is then placed in a watertight, secondary plastic container with a tightly sealed closure that cannot open during shipment.

CAUTION: Do Not Use Sealable Food Bags

- The specimen container and the secondary container must then be placed in a sturdy cardboard box protected with additional absorbent packing material.
- Etiologic agents and Biohazard Materials must be sent by first-class Mail, Priority Mail, Express Mail, UPS or FED-EX..

Leak-proof Formalin containers and adsorbent materials are available at:

Labsco at <http://www.labsco.info/default.aspx?Page=Home>

Consolidated Plastics 800-362-1000 at <http://www.consolidatedplastics.com/Natural-And-Clear-Wide-Mouth-Threaded-Jars-C1581.aspx>

Path-tec at <http://www.path-tec.com/products/productpages.htm>

Master List of Subcontractors

NOTE: Any test not listed in this Lab Users Guide is subject to subcontracting. Subcontracting of tests may also occur if a test or procedure normally conducted at Kord ADDL is not currently available. Submission of samples to Kord ADDL implies authorization to utilize testing by these laboratories. Any fee-based testing requires client preapproval. All toxicology samples are subcontracted to another division within the Tennessee Department of Agriculture.

Name of Subcontractor	Address	Phone	Tests Requested	Certification
NVSL	1800 Dayton Avenue USDA, APHIS Ames, IA 50010-9674	515-663-7212	Salmonella typing, TB culture, WNV, EEE, WEE, VEE, Fungal, AI, (MS, MG, HI), Brucellosis, PRV, EIA, CSF	ISO 17025
Plum Island Animal Disease Center	P.O. Box 848 Greeneport, LI Orient Point, NY 11944	631-323-3200	Foreign Animal Disease Confirmation	ISO17025
University of Tennessee-Knoxville Dept. of Pathobiology	2407 River Drive Room A201 Knoxville, TN 37996-4542	Phone 865-974-5673 Fax: 865- 974-5616	Formalin-fixed tissue	American Animal Hospital Association
Tennessee Department of Health	630 Hart LN Nashville, TN	615-262-6351	Rabies	External Proficiency Testing through Wisconsin State Dept. of Hygiene
Toxicology Section, Technical Services Division, Tennessee Department of Agriculture	Ellington Agriculture Center 440 Hogan Rd Nashville, TN 37220	615-837-5414	All routine toxicology samples	None, at direction of Director, Regulatory Services, Tenn. Dept. Agriculture
Dr. Phil Elzer LSU, Veterinary Immunology	111 Dalrymple Bldg Baton Rouge, LA 70803	225-578-4763	Swine Brucellosis	None, at direction of Tennessee AVIC

APPROACH TO DIAGNOSIS

The diagnostic laboratory depends on the referring veterinarian to submit quality samples which will provide optimal opportunity for the diagnosis of disease. Often we receive tissues to diagnose a specific disease entity when there are a number of other possibilities which may require additional tissues. Other times we receive tissues from animals that do not represent the disease situation. An organized, objective, and thorough approach is required to obtain the proper diagnosis. Listed below are steps to follow which aid in diagnosis.

- A. Obtain a good history; make careful and thorough clinical observations.
- B. Categorize the problem as to organs or organ systems that may be involved.
- C. Using the information obtained, form a broad list of possible diagnoses and rule out as many as possible.
- D. Narrow your list of possible diagnoses by additional clinical observations or “in house” laboratory procedures.
- E. Submit the history, clinical, and necropsy findings along with the proper tissues for diagnosing any one of the diseases listed in your differential. Follow the guidelines given on the following pages to determine which tissues are needed.
- F. If the condition is complex, or does not present a clear clinical picture, submit a broad assortment of tissues, including sections of major organs and grossly visible lesions.

ABORTIONS

- A. Diagnosis of the cause of abortion is difficult and complex.
- B. Submit tissues from multiple fetuses and placentas to increase the probability of diagnosis.
- C. Collect and submit the first of paired serum samples from the suspected aborting animal. The second serum sample should be collected and submitted in 2-3 weeks.
- D. If a toxic condition is suspected, submit samples of the aborting animal’s feed and water.
- E. If the fetus and placenta cannot be submitted, submit the following:
 1. FRESH TISSUE for bacterial culture and, virology
Stomach contents, placenta, liver, lung, spleen, kidney, and brain.
 2. FIXED TISSUE for histopathological examination
Placenta, Lung, liver, heart, adrenal, kidney, thymus, and brain.
 2. OCULAR FLUID (best) or eye for nitrates.

BACTERIOLOGY SUBMISSIONS

The Kord ADDL will not conduct any testing for food safety purposes or make any recommendations regarding safety or edibility of items for human consumption.

AEROBIC SPECIMENS FROM NECROPSIED ANIMALS

1. Collect all specimens as aseptically as possible. Liberal portions of each organ should be collected. If the outside of the specimen is accidentally contaminated, wash the specimen with clean tap water.
2. **Refrigerate** (wet ice packs) all specimens to prevent saprophytic overgrowth.
3. Collect observable lesions or suspected target organs.
4. For neonatal diarrhea, submit a tied off 4-5 cm segment of jejunum, ileum, and colon with the accompanying lymph nodes for culture of pathogenic bacteria.
5. Tissue specimens should be placed in individual leak-proof plastic bags and identified (use water-proof ink on bags).

MASTITIS MILK SPECIMENS

1. Wash udder to remove dirt and allow to dry.
2. Scrub teat end with alcohol soaked cotton and let it dry.
3. Samples should be collected in a sterile container immediately prior to regular milking without discarding any streams of milk (since the foremilk usually contains the greatest number of the infecting micro-organisms).

SWAB SUBMISSIONS - Collect samples aseptically and submit in commercial transport media.

ANAEROBIC AND MICROAEROPHILIC SPECIMENS

Note: The success of culture for anaerobic and microaerophilic organisms is heavily dependent on sample selection and shipment.

1. Sample should be taken from a living animal or a fresh carcass.
2. Specimens should be submitted in a transport media that limits or excludes air from the sample. Use a commercial anaerobic transport media swab.

MYCOLOGY (Fungal culture)

COLLECTION AND CARE OF SPECIMENS

1. Submit skin scrapings from the outer edges of a lesion and submit plucked (not cut) hairs.
2. Skin, hair, and nails should be shipped to the laboratory without refrigeration.
3. Submit internal organs or internal lesions suspected of fungal infection.
4. Internal specimens should be sent refrigerated (wet ice packs) and not frozen. Use whirl-paks and insulate.

RESULTS

Fungal isolations normally take longer than bacterial isolations; therefore, a tentative report may be made by the laboratory upon completion of direct microscopic examination of the specimen.

MYCOLOGY NOTE:

Blastomyces, Histoplasmosis, and Coccidioides are highly pathogenic dimorphic fungi that pose a significant risk to laboratory personnel. These organisms produce numerous arthrospores which are

readily disseminated in the culture process; therefore safety precautions in the laboratory preclude culture of these organisms at this time. These diseases are best diagnosed by serological methods, cytology or histopathology. These methods also provide a more rapid diagnosis than fungal culture.

USER'S GUIDE TO THE ANIMAL MICROBIOLOGY UNIT

The Animal Microbiology Unit of the Animal Disease Diagnostic Laboratory provides cultural examinations for a wide variety of diseases. Some of the most common are listed below.

TEST	SAMPLE
Abortion Screen	Placenta, fetal stomach contents, uterine contents, (includes culture ID for Trichomonas, Campylobacter, and Brucella)
Acid Fast Stain	Feces or intestine (for Johne's Mycobacterium paratuberculosis and Cryptosporidia).
Aerobic Culture	Fresh chilled tissue, urine, exudate, transtracheal wash
Anaerobic Culture	Fresh tissue, anaerobic culturettes, exudate
Antibiotic Sensitivity (disc diffusion, Vitek)	Performed on isolates recovered from specimens. Please request on accession form that you would like this test done.
Blood Culture	Blood submitted in blood culture bottle
Brucella abortus Culture	Fetal tissues, placenta, milk, lymph nodes
Brucella canis	Fetus, testicle, placenta, uncoagulated blood in a blood culture bottle, lymph nodes, vaginal discharge, milk, semen
Calf Scours	Feces or affected intestine
Campylobacter (Vibrio) Culture (bovine)	Preputial wash, vaginal fluid. CALL FOR PROTOCOL for protocol for submitting samples.
Campylobacteriosis	Affected intestine
Candida	Lesion, milk
Dermatomycosis	Lesion, hair, scales, fungal slants/trays
Dermatophilosis	Hair and scabs (please submit a good size sample)
Diarrhea/enteritis	Feces, affected intestine
Johne's (Mycobacterium paratuberculosis)	Feces (2 gram sample, Walnut-sized sample)
Listeriosis	Cerebellum, pons, medulla, fetus, uterine secretions
Lumpy Jaw (Actinomyces)	Exudate, lesion, sulfur granules
Mastitis (milk) culture	Milk submitted in whirl-paks or sterile tubes. Please NOTIFY the laboratory in advance if submitting more than 40 samples at a time.

Mycoplasma	Fresh chilled tissue, transtracheal wash, swab (may require 10-14 days for completion).
Paratuberculosis (Johne's).	Feces (2 gram sample, Walnut-sized sample)
Pinkeye (Moraxella bovis)	Culturette of affected eye. Sometimes difficult to isolate from normal flora.
Pneumonia	Lung (Please indicate if Mycoplasma, Hemophilus, or Rhodococcus is suspected.)
Salmonella	feces, feed, water, environmental samples
Streptococcus equi (Strangles)	Exudate from non-draining lesion
Trichomonas culture	Preputial wash, vaginal fluid, CALL FOR PROTOCOL before submitting samples

PROTOCOL FOR TRICHOMONAS and BOVINE CAMPYLOBACTER COLLECTION AND SUBMISSION IN CATTLE

TRICHOMONAS

Diagnosis of trichomoniasis is made when Trichomonas organisms are observed from the smegma or preputial flush samples of bulls, or the uterine/vaginal fluid from cows. All samples should be submitted in an InPouch™ TF pouch. These pouches may be purchased from Biomed Diagnostisics.¹ A positive diagnosis will be dependent on the efficacy of sample collection, and handling and processing. When a positive sample is found, at the client's request, it will be submitted to an approved laboratory for Polymerase Chain Reaction (PCR) to distinguish between T. foetus and other Trichomonas organisms. There will be a shipping charge and an additional charge for the PCR.

SPECIMEN COLLECTION: (The collection procedure is the same for both Campylobacter and Trichomonas; however, the transport media are different.)

MATERIALS REQUIRED:

- InPouch™TF pouches
- Disposable gloves
- Infusion pipette
- 20 ml syringes or
- Wooden applicator sticks/sterile cotton-tipped swabs

Male Animals: (A good site for directions on collecting samples²)

- Step 1 Use a separate pair of gloves and a separate collection device for each animal.
- Step 2 Clip the preputial hairs to about one-half inch and clean the preputial orifice.
- Step 3 If necessary; rinse out the preputial cavity with sterile saline (not water) to clean out mud and manure. This will help prevent contamination from non-pathogenic intestinal trichomonads and coliform bacteria.

- Step 4 Insert the sampling device (pipette inside tube) into the preputial opening to about the distal third of the preputial cavity. Advance the collecting pipette through and beyond the protecting tube to the preputial fornix.
- Step 5 Collect the sample by rapidly scraping the pipette back and forth in short strokes on the mucosa of the distal penis and fornix area while applying suction with a rubber bulb or syringe and massaging the glans penis through the sheath to move smegma into the pipette. Fifteen to thirty (15 – 30) strokes of the pipette are required to obtain an adequate sample.

Female Animals:

- Step 1 Use a separate pair of gloves and a separate collection device for each animal.
- Step 2 Clean debris from the vulva.
- Step 3 Immobilize the cervix per rectum and insert the sampling device into the anterior third of the vagina.

Advance the pipette gently to the floor of the vaginal fornix, and patiently aspirate mucus. This may take 20 – 60 seconds, due to the viscosity of mucus. Some persistence may be required to get a thick, slightly yellow material that tends to stick to the pipette

The specimen should not be refrigerated or frozen.

Inoculation of InPouch™

- Step 1 Remove the pouch from the bag, and manually express the liquid so that there is approximately 1 ml in the upper chamber.
- Step 2 Tear the pouch open at the notch just above the closure. Open the pouch by pulling the closure tape's middle tabs apart.
- Step 3 Bovine infusion pipette - Insert the specimen pipette tip into the liquid of the pouch's upper chamber and expel 1.0-1.5 ml of the sample into the pouch. If the collected material adheres to the wall of the pipette, rinse the pipette by flushing a small amount of the liquid medium back and forth into the pouch, minimizing the production of bubbles. This maintains an anaerobic environment.
If using a swab, insert the swab into the liquid of the pouch's upper chamber and press the tip of the swab between the fingers through the flexible walls of the pouch.
- Step 5 Express the media into the lower chamber and roll the pouch down until the tape is at the top of the label. Fold the wire tape's end tabs to lock the roll. Write the animal ID on the label.

For Campylobacter:

Since Campylobacter is microaerophilic, inoculate an Amies transport media tube by saturating a swab with the preputial wash and inserting the swab into the bottom of the tube. Try to avoid inoculation of air into the tube. If transport media is not available the sterile saline or lactated Ringer's solution may be submitted if the sample is being brought to the lab within 24 hours of collection.

Shipping Requirements:

The samples must be shipped to the laboratory by overnight express/one day delivery. The handling and shipping of the inoculated media samples is one of the most critical steps in Trichomonas diagnosis. The inoculated media should be kept at 65°F to 75°F. It is especially important to avoid overheating or freezing the samples. Ship the samples in insulated containers (**no ice**) that will protect the samples from extreme temperatures. It is very important to arrange shipping of the samples so that they arrive at the laboratory within 48 hours of collection. Be sure to not ship samples so they arrive at the laboratory over the week end, (i.e. do not ship on Friday) or on the day before a holiday.

¹BioMed Diagnostics, Inc.
1388 Antelope Road
PO Box 2366
White City, Oregon 97503
1(800) 964-6466
www.biomeddiagnostics.com

InPouch TM TF Test – Bovine	Cat # 11-1003	100 tests
InPouch TM TF Test – Bovine	Cat # 11-1001	20 tests

² <http://genex.crinet.com/page1475/campylobacter>

Pathology Services

NECROPSY SUBMISSIONS

Animals should be euthanized before submission to the laboratory.

Dead animals should be cooled as soon as possible after death.

1. Large animals should be thoroughly hosed down with cold water.
2. Birds, rabbits, and other fur bearing animals should be soaked in cold, soapy water, placed in a plastic bag, and refrigerated.

NOTE: Do not place animals in a plastic bag without prior cooling.

A cursory confirmatory examination or refusal of an animal for necropsy at any time will be at the discretion of the diagnostician 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 by a veterinarian or owner and therefore a necropsy is unwarranted. e.g. fractured leg, uterine prolapse, or chronic laminitis.

In all cases, the Kord ADDL diagnostician will determine the suitability of submitted animals, tissues, or other materials for testing and determining which tests are performed. When multiple animals are submitted representing a herd or flock problem, the Kord ADDL diagnostician will determine the number of animals to be necropsied.

Legal/Cruelty cases - 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.

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.

The Kord ADDL will not conduct any testing for food safety purposes or make any recommendations regarding safety or edibility of items for human consumption.

HISTOPATHOLOGY

COLLECTION AND CARE OF SPECIMENS

1. Accuracy of the diagnosis is directly proportional to the collector's ability to select the specimen that represents the lesion or disease process. Poor selection can result in inaccurate interpretation.
2. Specimens should include grossly observable lesions with a small amount of adjacent normal tissue.
3. Organ containing the lesion.
4. Tissue specimens should include the surface and all anatomical features; for example, specimens of the kidney must include cortex, medulla, and pelvis.
5. The entire brain should be removed and cut longitudinally on the midline into two equal portions; 1/2 should be submitted in 10% BNF for histopathology and 1/2 submitted fresh for other test procedures, as indicated. (Gross examination by an experienced pathologist is often necessary to locate focal lesions for further sectioning - random samples often miss important lesions.)
6. Specimens (except the brain) should be 1/2 cm to 1 cm thick. Specimens that are too thin cannot be properly trimmed for sectioning and those that are too thick decompose before they are fixed. (Formalin will penetrate approximately 3 mm on each side per day.)
7. Fixation must begin as soon as possible after a carcass is opened or a surgical specimen is procured.
8. Ten (10) parts of fixative should be used to fix one (1) part of tissue.
9. Formalin-fixed tissues are required for histopathology examination. Neutral buffered formalin formula is 900 cc of water, 100 cc 40% formaldehyde, and 5 gm calcium carbonate.
10. Intestinal specimens requiring critical examination of villi (i.e. rotavirus and coronavirus infection) require special handling. The preferred method is to tie off approximately 3 cm long segments of bowel and gently fill the segments with 10% neutral buffered formalin, using a needle and hypodermic syringe.
11. Skin and uterine biopsy specimens should be placed on a piece of tongue depressor or smooth cardboard; (Avoid paper; the subcutis or cut surface should be in contact with the wood or cardboard.)
12. The mouth of specimen containers should be wide enough to allow the tissue to drop into the bottle without touching the sides of the opening. Unfixed tissue can be easily forced into a jar that has an opening too narrow to allow removal following fixation without breaking the container.

SHIPMENT

1. Use wide mouth plastic or non-breakable bottles or vials with **leak-proof lids**.
2. Help improve our efficiency and turn-around-time. Avoid taping containers shut; it does not prevent leakage. (Refer to attached postal guidelines).
3. Pack the specimens with adequate padding to prevent breakage.
4. Avoid cramming large quantities of tissue into a small container.
5. Submit tissue in 10:1 ratio of fixative to tissue or fix the tissue and then transfer it to a smaller container with less formalin for shipment.

SUBMISSION FORM

1. Provide the requested information on the form.
2. Brief, concise, complete histories are required and aid in providing diagnoses and pertinent advice.
3. Please use black ink and **write or print legibly**.
4. List the tissues submitted, also the number of tumors. This will help insure that all submitted specimens are identified and examined.

CYTOLOGY

The laboratory offers cytologic and peripheral blood smear examinations. However, we **do not do clinical chemistries, CBC's or Differential blood counts**. These can best be accomplished by commercial or hospital laboratories.

I. Cytology

Cytologic aspirates are safe, easy, and often valuable. However, cytology does have its limitations. Material collected may not always represent the ongoing process. For example, large quantities of blood in an aspirate may represent part of the pathologic process or be due to the aspiration procedure. Insufficient cellular material in an aspirate may result when working with fibrous tissue such as fibrosarcomas. The quality of the sample strongly influences the diagnostic potential of cytology. Therefore, close attention must be made to slide preparation and handling. Ideally, preparations should be thin enough to visualize individual cells but cellularity must be sufficient for diagnosis. Cells should be handled gently to prevent destruction. Because fresh cells make the best preparation, slides should be prepared promptly.

A. Fine Needle Aspiration

1. Use a 25 gauge needle with 10-12 cc syringe and precleaned slides.
2. Make several vigorous aspirates from mass.
3. In order to avoid rupturing of cells, release suction pressure before removing the needle from mass. Often the specimen will be contained only in the hub of the needle.
4. After withdrawing the needle from the mass, remove the needle from the syringe. Then, fill the syringe with air, replace needle and use aspirated air to force cellular material onto slide.
5. Make a "squash" or "pull-apart" smear by covering the material on the slide with another slide, squashing the material on the slide with digital pressure and then pulling the slides apart. This must be done quickly as cytologic material often clots rapidly.
6. Please sent 3-4 unstained, air dried smears.

Note:

Lymph node aspirates must be handled gently. Lymphocytes are frequently damaged if shear force is applied to them. This is especially true in the case of malignant lymphoblasts. Slides should be squashed together by digital pressure and pulled apart vertically rather than horizontally to avoid shear force.

B. Imprints

Imprints can be made from solid tissue. A fresh surface should be blotted to remove the majority of surface blood. Several imprints per slide should be made. Material should not be smeared.

C. Scrapings

TISSUES of a fibrous nature are best sampled by scraping. A fresh surface is cut and then scraped using a clean scalpel or razor blade. The material is then gently spread across the slide.

D. Body fluids and washes

Slides from turbid fluid samples can be made in the same manner as peripheral blood slides. Clear or slightly turbid fluids should be centrifuged and the sediment spread on slides.

Smears must be made promptly after each collection. Cellular degeneration will be evident within 2 - 3 hours after collection.

E. Evaluation for blood parasites - Submit 2 unstained, air dried blood smears.

F. Only slides prepared at the time of collection will be examined. Do not submit only fluids or blood for microscopic evaluation.

Collection procedures can be reviewed in:

1. Rebar, A.H. (1978). Collection Techniques in Veterinary Cytology. In: *Handbook of Veterinary Cytology*. Pub. Ralston Purina Company, St. Louis, Missouri.
2. Crowell, R.L. and Tyler, R.D. Cutaneous and Subcutaneous Lesions: Masses, Cysts, Ulcers and Fistulous Tracts. In: *Diagnostic Cytology of the Dog and Cat*. Pub. American Veterinary Publication, Inc., Goleta, California.

PARASITOLOGY

- A. Limited parasitology is offered by the laboratory for diagnostic purposes on individual animals. For herd or group evaluations, samples should and will be combined.
- B. Evaluation for fecal parasites and protozoa such as cryptosporidia. Make sure enough intestinal content or feces is present in wet form (i.e. 3-4 grams, pecan-size)).

SEROLOGY

- A. **DON'T'S** in submission of serum samples and tissue specimens. Experience in the past has shown the following problems associated with shipping serum samples or tissue specimens to the laboratory (in order of decreasing occurrence):
1. Bacterial contamination.
 2. Serum not separated from clots.
 3. Chemical (detergent, disinfectants, etc.) contamination of serum causing toxicity to tissue culture or otherwise adversely affecting desired tests.
 4. Hemolysis.
 5. Insufficient quantity.
 6. Overheating or freezing of blood before serum extraction.
 7. Leaky stoppers.
 8. Broken containers.
 9. Improper container.
 10. Rotten tissue specimens.
- B. **DO'S** in submission of serum samples and tissue specimens:
The following requirements will help prevent the above listed problems from occurring.
1. Serum
 - a) Collect blood aseptically into a sterile dry tube, refrigerate immediately, allow to clot, centrifuge, and transfer serum aseptically into a second tube.
 - b) Serum must be fresh, clear, unhemolyzed, and uncontaminated.
 - c) Red top Vacutainers[®], B-D, or other non-EDTA/heparin sterile commercially available tubes are ideal.
 2. Label each tube (not stopper) with **tube number** and **vet code**. Be careful that writing will be legible upon arrival (use an indelible marker). Identify specimen in a way that will avoid confusion when results are reported. Avoid using animal names. Keep your own log of sample numbers to avoid duplication and confusion. Put small tubes in a box or tape to large cardboard so they will not be lost in the packing.
 3. Serum is preferred to be separated, by centrifugation, from clotted blood prior to submission for testing. Do not let whole blood freeze or expose to direct sunlight or high temperature before decanting serum.
 4. Submit at least 1.0 ml of serum for each test requested. Refrigerate the serum until shipment.
 5. Tissues should be collected aseptically and placed individually in well-labeled whirl-pak bags. Place individual whirl-pak bag into a single air tight container and refrigerate immediately.
 6. Swab samples for serological testing should be submitted in a small amount of sterile saline but only enough to moisten the swab and shipped in an insulated container with sufficient ice packs. **DO NOT send the swabs in the gel-type media that is used for bacteriology culture.**
 7. Refrigerated samples should be shipped with sufficient cool-pak bags and padding. Ship samples in a leak proof insulated container or in sturdy mailing carton which complies with postal or commercial carrier specifications (Page 10). Submit samples early in the week to avoid holding over the weekend by the carrier.
 8. A complete and legible form(s), (including a complete history), must accompany all serologic submissions. Be certain to indicate vaccination history. "All and Current" is not an appropriate vaccination history.
 9. All regulatory charts must include the submitting veterinarian's signature, vet code, and animal identification.
 10. Flock check serology should be based upon a sample size of 20-30 samples per flock. National Poultry Improve Plan (NPIP) testing may require larger number of samples.
 11. The laboratory is certified by the NVSL (National Veterinary Services Laboratory), USDA to perform Anaplasmosis ELISA tests, AGID tests for bluetongue, bovine leukemia virus and

equine infectious anemia, Johne's ELISA, Johne's polymerase chain reaction (PCR), leptospira microagglutination test, and pseudorabies serologic tests which include gB ELISA for screening, gp1 ELISA, latex agglutination test, and serum neutralization. The lab is also certified by the National Animal Health Laboratory Network, NVSL, USDA to perform polymerase chain reaction for diagnosis of avian influenza, classical swine fever, exotic Newcastle disease, and foot-and-mouth disease.

12. Equine Infectious Anemia (EIA or Coggins) serology requires at least two (2) ml of clear, fresh, unhemolyzed serum and takes 24 hours to complete. Retesting may be required. Each tube of serum submitted should be identified with **vet code** and the **tube number** corresponding to the tube number on the submission form. A **mane tag** is to be affixed to each horse tested and **tag number** recorded on the submission form. If additional tests other than EIA are requested, please, submit two samples. The submission form must be completely and correctly completed and signed by the submitting veterinarian.
 - a). **The lab cannot make any changes or fill in any missing information. All forms not properly completed will be returned for completion.**
 - b). **Samples received before 2PM will be set up for testing that day. Results are normally available in 3 business days. Samples received after 2PM will be tested the following day and results will normally be available in 4 business days from the date of drop-off.**
 - c). **Submit serum tubes in slotted blood boxes. These are supplied at no cost by calling 615-837-5231. Leave indicated information on recording.**

13. **Exports:** The submitting veterinarian is responsible for informing the laboratory of any special requirements (i.e., dilutions, type of test). If this information is not supplied, a delay on the required test result could happen.
For information on test regulations, call:
Federal - USDA, APHIS, VS, Veterinarian in Charge - (615)781-5310
State - Office of the State Veterinarian - (615) 837-5120

14. When calling for results, the laboratory must have the submitting veterinarian's name, the owner's name, the animal's name or ID, the sample number or ID, submitting date and bleeding date exactly as provided on the submission form.
15. Advance notice (7-10 days ahead) should be given (and will be greatly appreciated) when submitting a large number of samples.
16. For sero-diagnosis in the individual animal, paired sera are recommended to test for specific antibody: the first sample taken when the animal is initially examined (acute phase serum) and the second sample 2-4 weeks later (convalescent phase serum). A rise in antibody titer between the paired samples is a basis for a specific serologic diagnosis for a particular disease. The paired serum samples should be submitted together in order to obtain a better understanding of the diagnostic significance of titers as they relate to the clinic status of the animal.
17. If only a single serum sample can be obtained, it must be taken from a convalescent animal. An acutely ill animal is virtually devoid of antibody against the particular disease-causing agent.
18. If only acutely ill animals are present, swabs or tissues for isolation or culture of the causative agent should also be submitted.
19. **Submit serum tubes in slotted blood boxes. These are available at no cost by calling 615-837-5231. Leave indicated information on recording.**
20. If there are any questions regarding submission of samples, please call the Kord Lab at 615-837-5125.

Key to abbreviations for Serology Test Chart:

AGID - Agar gel Immunodiffusion

CA - Card Agglutination

CF - Complement Fixation
ELISA - Enzyme Linked Immunosorbent Assay
FA - Fluorescent Antibody (Direct)
HI - Hemagglutination Inhibition
IFA - Indirect Fluorescent Antibody
IHA - Indirect Hemagglutination
LA - Latex Agglutination
NVSL - National Veterinary Services Laboratory
PCR - Polymerase Chain Reaction
TA - Tube Agglutination

BVD EAR NOTCH SAMPLE COLLECTION & SUBMISSION REQUIREMENTS

MATERIALS AND EQUIPMENT NEEDED

1. Ear Notching tool that yields a 1 cm x 1 cm notch. Recommend purchasing two or more tools
 - **Nasco:** 1-800-558-9595. Order ID medium C0024N
 - **Dairy Health USA:** 1-800-276-7933. Order ID medium 440-31
 - **Stone Livestock Identification, Show Equipment:** 1-816-231-4020. Order ID medium 7125
2. Red Top Blood tube: The preferred tube is a **Vacutainer** 7 ml draw 16mm x 75mm collection tube with No Additives.
3. Shipping container that holds individual tubes in slots.
4. Submission Form from the Kord Animal Disease Laboratory (615-837-5125).
5. Disinfectant for rinsing notching tool: 10% bleach (e.g. 100 ml (3 oz.) bleach in 900 ml (27 oz.) water).
6. Clean rinse water: 3-5 gallon bucket. Change bucket water every 20-30 notches.
7. Disposable gloves and clean coveralls.

COLLECTION PROCEDURE

1. Label Red Top collection tubes with consecutive numbers (#1 through number in submission) and keep a list of the corresponding animal ID or tattoo numbers. This method of labeling significantly reduces the turnaround time in the laboratory and gets the results to you faster.
2. Dip notching tool in disinfectant, then **ALWAYS** rinse away disinfectant with copious quantities of clean water. **CAUTION: Residual disinfectant on the notching tool will yield false negative results, therefore thorough rinsing with clean water is required!!**
3. Collect an ear notch approximately 1 cm x 1 cm (3/8 in. x 3/8 in.) from a clean portion of the ear. Only fresh ear notch samples are acceptable for the antigen-capture ELISA test. The ear should not be cleaned with anything but water. Disinfectant of any kind (e.g. Novasan) can interfere with the test.
4. Place the fresh ear notch into a sterile, clean Vacutainer collection tube (no formalin, saline, bleach, other liquid, detergent residue, or separator gel). Do not use whirl-pac or any other type of plastic bags. **CAUTION: Collected ear notch must be free of contaminating dirt, feces, tattoo ink or BVD vaccine. Do not vaccinate or tattoo at the same time samples are taken.**
5. Send samples to the laboratory with sufficient ice packs to chill the specimen during shipment. Saline or other media must **NOT** be added to the tube containing the ear notch. Likewise, please do not use any fixative (e.g. Formalin).

6. Notify the laboratory in advance prior to shipment regarding the number of samples and when they will be sent. This allows us to have adequate supplies for testing and to arrange staffing to do the testing.

SEROLOGY TEST CHART

TEST	SPECIMEN	SHIPPING CONDITION	TYPE OF TEST	DAYS SET UP	SPECIAL COMMENTS (Submit serum in blood boxes)
Avian					
Avian Influenza	Serum	Refrigerated	AGID,PCR Strip-test	M,Tu,W, F	Requires at least 1 ml of serum
Chlamydia	Spleen, liver, lung, air sac, conjunctival swab	Refrigerated	FA	M-F	Reported as positive or negative
Mycoplasma gallisepticum	Serum	Refrigerated	ELISA, HI	Tu, Th	Requires at least 1 ml of serum.
	Trachea swab	Refrigerated		Special request required.	Submit in appropriate transport media.
Mycoplasma synoviae	Serum	Refrigerated	Plate test, HI	Tu, Th	Requires at least 1 ml of serum
	Trachea Swab	Refrigerated		Special request required.	Submit in appropriate transport media.
Canine					
Blastomycosis	Serum	Refrigerated	AGID	M-F	
Brucella canis	Serum	Refrigerated	CA	M-F	Samples are screened by the card test. Positives will not be reported until confirmed by the tube test.
		Refrigerated	TA	M,Tu,W, F	Samples run at 1:50, 1:100, 1:200 dilutions. Titers: 1:50 - Suspicious ≥1:100 – Positive
Calicivirus	Serum	Refrigerated	IFA	M-F	IgG, IgM determination. Samples run at 1:10, 1:50, 1:250, 1:1250 dilutions..

TEST	SPECIMEN	SHIPPING CONDITION	TYPE OF TEST	DAYS SET UP	SPECIAL COMMENTS
Distemper Virus	Lung, kidney, spleen, urinary bladder, brain, stomach, liver, blood smear	Refrigerated	FA	M-F	Reported as positive or negative
	Serum	Refrigerated	IFA	M-F	IgG, IgM determination. Samples run at 1:10, 1:50, 1:250, and 1:1250 dilutions..
Ehrlichia canis	Serum	Refrigerated	IFA	M-F	Sample is tested at 1:10, 1:50, 1:250, and 1:1250 dilutions.
Herpesvirus	Lung, liver, kidney, spleen, lymph node	Refrigerated	FA	M-F	Reported as positive or negative.
	Serum	Refrigerated	IFA	M-F	IgG, IgM determination. Samples run at 1:10, 1:50, and 1:1250 dilutions.
Histoplasmosis	Serum	Refrigerated	AGID	M-F	
Infectious Canine Hepatitis	Serum	Refrigerated	IFA	M-F	IgG, IgM determination. Samples run at 1:10, 1:50, 1:250, and 1:1250 dilutions. .
Lyme Disease	Serum	Refrigerated	IFA	M-F	Samples run at 1:64, 1:128, 1:256, 1:512 dilutions
Neospora caninum	Brain, lung, liver, kidney, muscle, skin	Refrigerated	FA	M-F	Reported as positive or negative
	Serum	Refrigerated	IFA	M-F	Sample tested at 1:50..

TEST	SPECIMEN	SHIPPING CONDITION	TYPE OF TEST	DAYS SET UP	SPECIAL COMMENTS
Parvovirus	Intestine (jejunum, ileum), spleen, mesenteric lymph node	Refrigerated	FA	M-F	Reported as positive or negative IgG, IgM determination. Samples run at 1:10, 1:50, 1:250, and 1:1250 dilutions.
	Serum	Refrigerated	IFA	M-F	
Rocky Mountain Spotted Fever (RMSF)	Serum	Refrigerated	IFA	M-F	Samples run at 1:64, 1:128, 1:256, 1:512 dilutions
Equine					
Equine Infectious Anemia (EIA)	Serum	Refrigerated	AGID	M-F	Complete EIA form (VS Form 10-11). Submitting veterinarian's signature is required. Samples received before 2PM will be set up for testing that day. The test takes 24 hours. Submit tubes in slotted blood boxes (Available at no cost)
Equine Rhinopneumonitis Virus (ERV)	Lung, liver, spleen, fetal tissues	Refrigerated	FA	M-F	Reported as positive or negative
	Serum	Refrigerated	SN	M,Th,F	Titers begin at 1:4.1:8, 1:16, 1:32, 1:64, 1:128: 1:256
Potomac Horse Fever	Serum	Refrigerated	IFA	M-F	Samples run at, 1:80 and 1:160 dilutions.
West Nile Virus	Serum	Refrigerated	ELISA	W	Reported as positive or negative
Feline					
Chlamydia	Conjunctival smear, nasal swab, lung	Refrigerated	FA	M-F	Reported as positive or negative
Feline Calicivirus	Serum	Refrigerated	FA	M-F	Samples are tested for IgG titers at 1:10, 1:50, 1:250 and 1:1250 dilutions

TEST	SPECIMEN	SHIPPING CONDITION	TYPE OF TEST	DAYS SET UP	SPECIAL COMMENTS
Feline Herpes virus	Nasal swab, conjunctival swab, tonsil, trachea, lung	Refrigerated	FA	M-F	Reported as positive or negative
	Serum	Refrigerated	IFA	M-F	Samples are tested for IgG titers at 1:10, 1:50, 1:250 and 1:1250
Feline Immunodeficiency Virus (FIV)	Serum	Refrigerated	IFA	M-F	Sample is tested at 1:25 and 1:50 dilution
Feline Infectious Peritonitis (FIP)	Affected tissues (liver, spleen, lymph nodes)	Refrigerated	FA	M-F	Reported as positive or negative
	Serum, pleural or peritoneal fluid	Refrigerated	IFA	M-F	Sample is tested at 1:6400 dilution
Feline Leukemia	Blood smears	Slide mailers	IFA	M-F	Submit at least 2 smears. Thick smears are not acceptable. Do not use old or used slides.
	Serum	Refrigerated	IFA	M-F	Sample is tested at 1:25 and 1:50 dilutions.
Panleukopenia	Small intestine, lymph node, spleen, aborted fetus	Refrigerated	FA	M-F	Reported as positive or negative
	Serum	Refrigerated	IFA	M-F	Samples are tested for IgG titers at 1:10, 1:50, 1:250 dilutions.
Toxoplasmosis	Serum	Refrigerated	IFA	M-F	

TEST	SPECIMEN	SHIPPING CONDITION	TYPE OF TEST	DAYS SET UP	SPECIAL COMMENTS
Porcine					
E.coli Pilus	Small intestine, feces	Refrigerated	IFA	M-F	Test for K88, P987 serotypes.
Leptospirosis	Serum	Refrigerated	MA	M-F	Test for 6 serovars - canicola, grippotyphosa, hardjo, icterohemorrhagiae, pomona, and bratislava. Samples tested at 1:100, 1:200, 1:400, 1:800 dilutions.
	Kidney	Refrigerated	FA	M-F	.
Porcine Parvovirus	Fetal tissues	Refrigerated	FA	M-F	Reported as positive or negative
	Fetal serum or fetal fluid	Refrigerated	IFA	M-F	Samples run at 1:10, 1:50, and 1:250 dilutions. A single serum sample from the dam is of little value since the breeding herd is often seropositive.
Porcine Respiratory and Reproductive Syndrome (PRRS)	Serum	Refrigerated	IFA	M-F	Sample is tested at 1:20 dilution.
Pseudorabies	Brain, Lung, Tonsil, Kidney	Refrigerated	FA	M-F	Reported as positive or negative
	Serum	Refrigerated	SN	M,Tu,W, F	Samples must be clear, unhemolyzed serum that is poured off clot.
	Serum	Refrigerated	LA		Reported as positive or negative
	Serum	Refrigerated	ELISA		Reported as positive or negative
Rotavirus	Feces, intestine	Refrigerated	ELISA	Tu and F	Reported as positive or negative.
Swine Influenza	Nasal secretions, trachea, lung	Refrigerated	FA	M-F	Reported as positive or negative

TEST	SPECIMEN	SHIPPING CONDITION	TYPE OF TEST	DAYS SET UP	SPECIAL COMMENTS
Transmissible Gastroenteritis (TGE)	Intestine (jejunum, ileum)	Refrigerated	FA	M-F	Reported as positive or negative
Ruminant					
Anaplasmosis	Serum	Refrigerated	CA	M-F	Samples must be at least 48 hours old from time of collection to time test conducted. Time of collection must be written on submission form. Result is reported as positive or negative. For export purposes. Advance notification (at least 1 week ahead) is required.
	Serum	Refrigerated	ELISA	M-F	
Bluetongue	Serum	Refrigerated	AGID	M-F	Reported as positive or negative.
Bovine Leukosis (BLV)	Serum	Refrigerated	AGID	M,Tu,W,F	Reported as positive or negative
Bovine Respiratory Syncytial Virus (BRSV)	Lung, bronchial lymph node	Refrigerated	FA	M-F	Reported as positive or negative
	Serum	Refrigerated	IFA	M-F	Samples run at 1:50 dilution.
Bovine Viral Diarrhea (BVD)	Lung, intestine, turbinate, trachea, swabs from lesions, fetal organs	Refrigerated	FA	M-F	Reported as positive or negative
	Serum	Refrigerated	SN	Tu,W,Th,F	Titers: 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256

TEST	SPECIMEN	SHIPPING CONDITION	TYPE OF TEST	DAYS SET UP	SPECIAL COMMENTS
Bovine Viral Diarrhea Persistent Infection (BVDPI)	Serum, ear notch	Refrigerated	ELISA	M-F	Instructions for ear notch submission
Caprine Arthritis-Encephalitis (CAE)	Serum	Refrigerated	AGID	M-F	
Chlamydia	Lymph node, tissues of aborted fetus, joint fluid	Refrigerated	FA	M-F	Specifically request Chlamydia.
Clostridium	Gangrenous muscle, liver, lung	Refrigerated	FA	M-F	Test for Clostridium chauvoei and novyi
Coronavirus	Intestine (spiral colon, ileum, and jejunum)	Refrigerated	FA	M-F	Reported as positive or negative
E.coli Pilus	Small intestine	Refrigerated	IFA	M-F	Test for K99 serotype. Currently unavailable due to lack of commercial reagents.
Epizootic Hemorrhagic Disease (EHD)	Serum	Refrigerated	AGID	M-F	Reported as positive or negative
Infectious Bovine Rhinotracheitis (IBR)	Lung, trachea, turbinate, aborted fetal tissues	Refrigerated	FA	M-F	Reported as positive or negative
	Serum	Refrigerated	SN	Tu,W,Th,F	Titers: 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256
Johne's Disease (Mycobacterium paratuberculosis)	Serum	Refrigerated	ELISA PCR	Thursday	

TEST	SPECIMEN	SHIPPING CONDITION	TYPE OF TEST	DAYS SET UP	SPECIAL COMMENTS
Leptospirosis	Kidney	Refrigerated	FA	M-F	.
	Serum	Refrigerated	MA	M-F	Test for 6 serovars - canicola, grippityphosa, hardjo, icterohemorrhagiae, pomona, and bratislava. Samples tested at 1:100, 1:200, 1:400, 1:800
Listeria	Serum	Refrigerated	CA	M-F	Test for Type 1 and Type 4 serotypes. Screened at 1:20. Titers: 1:20, 1:40, 1:80, 1:160
Neospora caninum	Brain, lung, kidney, liver	Refrigerated	FA	M-F	Reported as positive or negative
	Serum	Refrigerated	IFA	M-F	Serum run at 1:400 dilution.
Ovine Progressive Pneumonia (OPP)	Serum	Refrigerated	AGID	M-F	
Rotavirus	Feces, intestine (spiral colon, ileum, and jejunum)	Refrigerated	ELISA	Tu and F	. Reported as positive or negative

TOXICOLOGY

For suspected poisoning cases, samples will be subcontracted for toxicology testing. For routine toxicology testing, samples are submitted to the Toxicology Section, Technical Services Division, Tennessee Department of Agriculture and submission to Kord ADDL implies authorization for subcontracting to this laboratory. The client is responsible for any costs associated with toxicology testing. For samples sent to another laboratory for toxicology testing at the client's request, the client is responsible for the cost. When poisoning is suspected, please notify the laboratory at the time of submission.

Test	Minimum Sample Required	Comments:	
Aflatoxin	1 lb. feed (grain)	No hay or silage	
Anticoagulant (screen)	5 ml serum, blood, plasma 10 g bait 25 g stomach contents 150 g liver	Includes:	Warfarin, Bromadiolone, Difenacoum, and Brodifacoum Avoid submitting samples in medicine bottles Do not freeze sample
Arsenic	10 g liver and kidney 10 ml urine 10 ml blood 10 g stomach contents 100 ml water 50 g soil 10 g feed	Liver or kidney is the preferred sample from a dead animal.	
Brodifacoum	See Anticoagulant		
Bromadiolone	See Anticoagulant		
BUN	2 ml ocular fluid 2 ml serum or plasma		
Calcium	2 ml ocular fluid 2 ml serum		

Toxicology

Test	Minimum Sample Required	Comments:
Carbamate (Pesticide Screen)	10 g stomach contents 50 g rumen contents 10 g bait 1 lb. feed	Avoid submitting samples in medicine bottles. Avoid plastic containers. Glass is preferred.
Copper	5 ml serum 10 g liver or kidney 1 lb. feed	Liver is preferred over kidney.
Cyanide	Plants with cyanogenetic potential i.e. sorghums, sudan grass, corn 1 lb. dry plants 5 lb. wet plants 50 g muscle (heart)	Samples should be quick frozen as soon as possible for shipment to the lab.
Dicumarol	Feeds containing sweet clover 5 lb. wet plants 1 lb. dry plants 10 -20 ml blood	
Difenacoum	See Anticoagulant	
Drug Screen *See end, for list of drugs included in screen.	10 - 20 ml serum, whole blood (plasma) 10 - 20 ml urine 10 g bait, pills 10 g stomach contents	Avoid submitting samples in medicine bottles. Urine is the preferred sample.
Ethylene glycol	5 ml urine 5 ml serum, plasma 10 g stomach contents 10 g bait	
Fumonisin	1 lb. feed	No hay or silage.
Gossypol, free	1 lb. feed containing cottonseed	
Iron	10 ml serum	

Toxicology

TEST	SPECIMEN	SHIPPING CONDITION
Lead	5 ml blood, (EDTA, heparin) 10 g liver and kidney 10 ml water 10 g stomach contents	Heparin is preferred. Submit both liver and kidney.
Magnesium	2 ml ocular fluid 2 ml serum	
Metal Screen	5 ml serum or plasma (per screen) 10 g liver, kidney 1 pt water	Submit serum samples in royal blue top vacutainer tubes for trace metal analysis if zinc is included in screen. Call lab for details prior to testing.
Monensin	1 lb. feed	
Nitrate (qualitative)	2 ml ocular fluid	
Nitrate (quantitative)	1 lb. dry forage 5 lb. wet forage 1 pt water	
Ochratoxin	1 lb. feed (grain)	No hay or silage.
Organochlorines (pesticide screen)	10 g stomach contents 20 g rumen contents 10 g liver or fat 10 ml blood	Avoid submitting samples in medicine bottles. Avoid plastic containers. Glass is preferred.
Organophosphates (pesticide screen)	10 g stomach contents 20 g rumen contents 1 lb. feed	Avoid submitting samples in medicine bottles. Avoid plastic containers. Glass is preferred.
pH	1 lb. silage	
Potassium	2 ml ocular fluid 2 ml serum	
Protein, Total	.5 ml serum	Should not be hemolyzed or lipemic.
Selenium	10 g liver or kidney 10 ml whole blood 10 ml serum	Whole blood is preferred over serum.

TEST	SPECIMEN	SHIPPING CONDITION
Sodium	2 ml ocular fluid 2 ml serum 2 ml urine 1 lb. feed	
Strychnine	10 g stomach contents 10 ml urine 5 ml serum 50 g kidney (more if available)	Avoid submitting samples in medicine bottles.
T-2	1 lb. feed (grain)	No hay or silage.
Urea	1 lb. feed	
Vomitoxin	1 lb. feed (grain)	No hay or silage.
Warfarin	See Anticoagulants.	
Zearalenone	1 lb. feed	No hay or silage.
Zinc	10 g liver or kidney 5 ml serum 10 g stomach contents 1 lb. feed 5 ml serum	Serum sample should be in Royal blue top vacutainer tube for trace metal analysis.

*Drug Screen

Drug Screen A					Drug Screen B		
Acetaminophen	Cocaine	Meperidine	Morphine	Quinine	Amobarbital	Ethinamate	Phenytoin
Amitriptyline	Codeine	Methamphetamine	Nortriptyline	Strychnine	Aprobarbital	Glutethimide	Secobarbital
Amphetamine	Diazepam	Methadone	PCP	Trifluopromazine	Barbital	Pentobarbital	
Caffeine	Doxepin	Methaqualone	Propoxyphen	Trimeprazine	Butabarbital	Phenobarbital	
Chlorpromazine					Diazepam		

Call the lab if drug of interest is not listed.

10 gram of tissue is approximately equal in size to a golf ball.

VIROLOGY

1. Rabies: The Tennessee Department of Health is responsible for Rabies testing in Tennessee. The Kord Animal Disease Diagnostic Laboratory does not do rabies examinations. The brain or head (whole bodies not accepted) from an animal suspected of having rabies may be submitted to the regional public health laboratory by the responsible county authority.. This may be the Animal Control Unit in larger cities or the county Health Department Rabies Control Officer. Addresses of regional Tennessee Department of Health laboratories are under Other Sources of Help in Tennessee.
2. Collect the specimens during the early stage of clinical diseases (the acute phase).
3. Collect swabs or tissue specimens as aseptically as possible.
4. Place the specimens individually in well labeled sterile test tube (swab) or whirl-pak bag.
5. Refrigerate or freeze the samples immediately after collection.
6. Do not let EDTA-blood freeze or be exposed to direct sunlight or high temperature prior to submitting for bovine viral diarrhea (BVD) virus isolation from buffy coat. Keep the EDTA-blood refrigerated.
7. Specimens should be sent to the laboratory as quickly as possible to arrive within 24-48 hours.
8. Ship refrigerated or frozen samples with sufficient cool-paks. Use leak proof insulated styrofoam containers or sturdy mailing cartons, which comply with postal or commercial carrier specifications, to ship the samples. Submit samples early in the week to avoid holding over weekend by the courier. Best is overnight shipping.
9. Brief information concerning history of disease, number, and age of animals affected, vaccination, etc. should accompany the samples.
10. When using "culturette" swabs, do not break the ampule as it may contain media which is inhibitory to some viruses.
11. Swab samples for virus isolation should be submitted in a small amount of sterile saline, but only enough to moisten the swab. The specimen should be shipped in an insulated container with sufficient ice packs. **DO NOT send the swab in the gel-type transport media that is used for bacteriology culture.**
12. Virus isolation is performed on Tuesday, Wednesday, and Friday. It requires 7 to 14 days for completion.
13. Isolation of a microorganism does not confirm that it is the causative agent of the illness. Failure to isolate the suspected etiologic agent does not rule out the suspected disease.

VIROLOGY TEST CHART

TESTS	SPECIMEN	SHIPPING CONDITION	TYPE OF TEST	SPECIAL COMMENTS
<u>Canine</u>				
Canine Adenovirus	Lung, liver, kidney, spleen, lymph node	Refrigerated	VI	
Canine Calicivirus	Oropharyngeal swab, trachea, lung, kidney, intestine	Refrigerated	VI	
Canine Herpesvirus	Lung, liver, kidney, spleen, lymph node	Refrigerated	VI	
Canine Parvovirus	Small intestine, spleen, mesenteric lymph node	Refrigerated	VI	
<u>Equine</u>				
Equine Rhinopneumonitis Virus (ERV)	Lung, trachea, nasal swab, fetal organs	Refrigerated	VI	
<u>Feline</u>				
Feline Calicivirus	Oropharyngeal swab, nasal swab, lung, trachea	Refrigerated	VI	
Feline Herpesvirus (Feline Rhinotracheitis)	Nasal swab, tonsil, trachea, lung	Refrigerated	VI	
Feline Panleukopenia (Feline Distemper)	Small intestine, spleen, lymph node, fetal tissue	Refrigerated	VI	

TESTS	SPECIMEN	SHIPPING CONDITION	TYPE OF TEST	SPECIAL COMMENTS
<u>Porcine</u>				
Porcine Respiratory and Reproductive Syndrome (PRRS)	Lung, spleen, liver, tonsil, fetal tissues	Refrigerated	VI	
Porcine Parvovirus	Fetal tissues, spleen, lymph nodes	Refrigerated	VI	
Pseudorabies	Brain, tonsil, lung, spleen	Refrigerated	VI	
<u>Ruminant</u>				
Bovine Respiratory Syncytial Virus (BRSV)	Lung, trachea	Refrigerated	VI	Specifically request BRSV isolation.
Bovine Viral Diarrhea (BVD) /Mucosal Disease	Buffy coat (EDTA blood), lung, intestine, spleen, fetal tissues	Refrigerated	VI	Please notify the laboratory regarding herd screening.
Infectious Bovine Rhinotracheitis (IBR)	Lung, trachea, fetal tissues	Refrigerated	VI	
Poxvirus	Skin lesion	Refrigerated	VI	

Key to abbreviations:

VI - Virus isolation

OTHER SOURCES OF HELP IN TENNESSEE

State Veterinarian
Regulatory/Interstate shipping/Health certificates
Dr.Charles Hatcher – State Veterinarian
Phone (615) 837-5120

West Tennessee Animal Diagnostic Laboratory
UT-Martin
Phone (731)881-7952
Days and weekends

College of Veterinary Medicine, U.T. Knoxville
Necropsy – Days
Necropsy – after hours
Phone (423) 974-8387
Phone (865) 974-5673
Phone (865) 974-5701

TWRA (Tennessee Wildlife Resources Agency)
Phone (615) 781-6500

Tennessee Department of Health
<http://health.state.tn.us/contact.htm>
Drs. John Dunn and Rand Carpenter,
Veterinary Epidemiologists/Rabies information
Phone (615) 741-3111
Phone (615) 741-7247

USDA-APHIS - Veterinary Services
“The Feds”
Phone (615) 781-5310

State/Federal Brucellosis Records
(Brucellosis Results)
Phone (615) 837-5120

County Extension Offices – multiple locations

Rabies Laboratories (Specimen Mail, U.S. Postal Services)

Nashville
Tennessee Department of Health
Laboratories Services
630 Ben Allen Rd.
Nashville, TN 37247
or (if mailing) P.O. Box 305130
Nashville, TN 37230-5130
Phone (615) 262-6350

Jackson Branch Rabies Laboratory
Tennessee Department of Health
295 Summar Avenue
Jackson, TN 38302-0849
Phone (901) 423-6600

Knoxville Branch Rabies Laboratory (U.S. Postal)
East Tennessee Regional Office
1522 Cherokee Trail
P.O. Box 59019
Knoxville, TN 37950-9019
Phone (423) 549-5201

Animal Poison Control Center - ASPCA

For any animal poison-related emergency, 24 hours a day, 365 days a year. If you think that your pet may have ingested a potentially poisonous substance, call **(888) 426-4435**. A \$60 consultation fee may be applied to your credit card.

Humans – Tennessee Poison Control Center

Tennessee Poison Center (TPC) provides immediate treatment advice for poison emergencies by calling the *Poison Help* hotline at [1-800-222-1222](tel:1-800-222-1222). Available to help 24 hours a day, 7 days a week. TPC also provides information about poisons and poison prevention.

Tennessee Poison Center can help you with questions about: household products, chemicals at work or in the environment, drugs (prescription, over-the-counter, herbal and illegal), snake and spider bites, and chemical terrorism

For life-saving treatment advice about any kind of poison, call [1-800-222-1222](tel:1-800-222-1222) first. A specially trained nurse, pharmacist or doctor will help. All calls are free and confidential.

Reportable Diseases

The reporting of evidence of certain animal diseases is a requirement under Standards for Accreditation of Veterinarians in Tennessee and other states. Such reporting is also required by State law in Tennessee.

Most important, perhaps, is the veterinarians professional responsibility to report these diseases properly to insure that appropriate control measures may be instituted. Reportable diseases in general include all diseases for which control or eradication programs are in effect, and all foreign diseases (not known to exist in this country).

Reportable diseases currently include but are not limited to:

Avian

Fowl Plague
Fowl Typhoid
Pullorum Disease
Velogenic Viscerotropic Newcastle Disease

Bovine

Anthrax
Brucellosis
Rabies
Scabies (psoroptic)
Screwworms
Tuberculosis
Vesicular Diseases

Porcine

African Swine Fever
Anthrax
Brucellosis
Hog Cholera
Pseudorabies
Rabies
Screwworms
Vesicular Diseases

Canine and Feline

Rabies

All Species - Rabies

Suspected or known rabies infection should also be reported to local public health authorities. Reporting should include telephone or written notice to the State Veterinarian's office and submission of samples to the appropriate laboratory.

Equine

Anthrax
Equine Infectious Anemia
Piroplasmiasis
Rabies
Screwworms
Vesicular Diseases
Viral Encephalitis

Sheep & Goats

Anthrax
Bluetongue
Rabies
Scabies
Scrapie
Screwworms
Vesicular Diseases

**TENNESSEE DEPARTMENT OF AGRICULTURE
DIVISION OF REGULATORY SERVICES
KORD ANIMAL DISEASE DIAGNOSTIC LAB**

SURGICAL BIOPSY – Submission Form

DATE _____ VET CODE _____

CLINIC _____

VETERINARIAN _____

STREET _____

CITY _____ STATE _____ ZIP _____

PHONE () _____ FAX () _____

OWNER _____

STREET _____

CITY _____ STATE _____ ZIP _____

COUNTY _____ PHONE () _____

Animal ID or Name _____

PLEASE CIRCLE BELOW WHERE APPLICABLE:

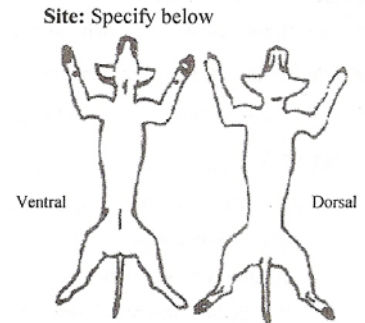
EXAM REQUESTED: Histopathology Cytology Culture Sensitivity FA Serology Toxicology Virology Other _____

SPECIES: avian bovine canine caprine equine feline ovine porcine other _____

BREED: _____ **SEX:** female/spayed male/neuter **AGE:** days _____ months _____ years _____

SAMPLE (s) SUBMITTED: _____

BIOPSY:	SIZE:	<1-5 cm diameter	1-5 cm	>5 cm
	REMOVAL:	incisional	excisional	
	CHARACTER:	discrete	infiltrative	
	CONSISTENCY:	cystic firm hard	soft other _____	
	DISTRIBUTION:	focal symmetrical	multifocal non-symmetrical	
	PRURITIC:	Yes	No	
	SEASONAL:	Yes	No	



Duration: _____ **Number of tissues:** _____

History: _____

FOR LAB USE ONLY

**KADDL PATH.206-03A
Rev.1, April 2011
Page 1 of 1**

Bacteriology _____ Cytology _____

Immunology _____

Direct FA _____

Virology _____ Parasitology _____

Toxicology _____

Retained: Brain Fresh Intestine Feces Serum, Blood

Stomach Contents None _____

Remarks _____

Tissues (#) _____ Trimmed by _____

Necropsy _____ Decal _____ Mailed _____

U.S. Postal Service Address:
C. E. Kord Animal Disease Laboratory
P.O. Box 40627 Melrose Station
Nashville, TN 37204-0627
Telephone # 615-837-5125
FAX # 615-837-5250



Overnight Delivery Address:
C. E. Kord Animal Disease Laboratory
Ellington Agriculture Center
440 Hogan Rd
Nashville, TN 37220

Need forms (?) Yes _____ No _____

TENNESSEE DEPARTMENT OF AGRICULTURE
DIVISION OF REGULATORY SERVICES
KORD ANIMAL DISEASE DIAGNOSTIC LAB

FOR LAB USE ONLY

KADDL PATH.206-03B
Rev.0, April 2011
Page 1 of 1

Fresh-Fixed Tissue - SUBMISSION FORM

DATE _____ VET CODE _____

CLINIC _____

VETERINARIAN _____

STREET _____

CITY _____ STATE _____ ZIP _____

PHONE () _____ FAX () _____

OWNER _____

STREET _____

CITY _____ STATE _____ ZIP _____

COUNTY _____ PHONE () _____

Animal ID or Name _____

PLEASE CIRCLE BELOW WHERE APPLICABLE:

Bacteriology _____ Cytology _____

Immunology _____

Direct FA _____

Virology _____ Parasitology _____

Toxicology _____

Retained: Brain Fresh Intestine Feces Serum, Blood

Stomach Contents None _____

Remarks _____

Tissues (#) _____ Trimmed by _____

Necropsy _____ Decal _____ Mailed _____

EXAM REQUESTED: Histopathology Cytology Culture Sensitivity FA Serology Toxicology Virology Other _____

SPECIES: avian bovine canine caprine equine feline ovine porcine other _____

BREED: _____ **SEX:** female/spayed male/neuter **AGE:** days _____ months _____ years

SAMPLE (s) SUBMITTED: _____

SPECIMEN(s) SUBMITTED:

Fixed: Brain Heart Stomach Intestine Liver Lung Spleen Kidney Other _____

Fresh: Brain Heart Stomach Intestine Liver Lung Spleen Kidney Other _____

Serum Blood Ocular Fluid Urine Stomach contents Feed Other: _____

TEST FOR: _____

HISTORY (Clinical signs, management practices, ration, history of disease, etc.)

Duration of illness: _____

Treatments: Antibiotics Corticosteroids Fluids Other _____

Vaccination status: None Up to Date Unknown

Vaccinated for: _____

Number of Animals in Group: _____ Number of Sick _____ Number of Dead _____

**OWNER/SUBMITTOR QUESTIONNAIRE
C. E. KORD ANIMAL DISEASE DIAGNOSTIC LAB**

Date of Submission _____

VETERINARIAN

Name _____

OWNER

Name _____

Street _____

City _____

County _____ Zip _____

Phone _____

Animal ID or Name _____

FOR LAB USE ONLY

Bacteriology _____ Cytology _____

Immunology _____

Direct FA _____

Virology _____ Parasitology _____

Toxicology _____

Retained: Brain Fresh Intestine Feces Serum, Blood

Stomach Contents None _____

Remarks _____

Tissues (#) _____ Trimmed by _____

Necropsy _____ Decal _____ Mailed _____

Telephone _____ Faxed _____

Please answer all the following questions.

1. What is being submitted? (i.e. cow, calf, etc.) _____
2. Age _____ Sex _____ Breed _____
3. When did it die? _____
4. How many animals have died in the past 2 weeks? _____
5. How many are currently sick? _____
6. How many animals are at this location? _____
7. Where is the animal kept? _____
8. What is the normal diet animal receives? (i.e. pasture, hay, type of grain, commercial food, etc.)

9. Any recent changes, if so, describe. _____

10. What is the source of drinking water? (i.e. city, well, pond, etc.) _____

11. Describe the illness prior to the animal's death. (i.e. appetite changes, drinking, scours, cough, duration of illness, etc.) _____

12. What treatments did the animals receive? (i.e. antibiotics, wormers, other) _____

13. Does the animal have access to any poisons? (i.e. garage, barns, dump site, woods, shrub trimmings, etc.) If so, describe. _____

14. For which diseases is the animal(s) vaccinated? (i.e. BVD, Lepto, Blackleg, etc., Distemper, Parvovirus, etc.) _____

15. When and where did you obtain the animal? _____

**TENNESSEE DEPARTMENT OF AGRICULTURE
C. E. KORD ANIMAL DISEASE LABORATORY, REGULATORY SERVICES DIVISION**

OWNER'S RELEASE FORM

There are some things that you, as the owner or owner's agent, should know and understand about the Kord Animal Disease Diagnostic Laboratory (KADDL):

1. This is a diagnostic laboratory in which dead animals are examined to determine the cause of death or disease. This is not a clinic in which animals are treated and returned to the owner.
2. Animals submitted to the KADDL will not be returned to their owners or agents.
3. All animals examined at the KADDL will be cremated or disposed of in an appropriate manner.
4. You should not enter the room in which postmortem examinations are done so that you will not track disease problems back to your farm or premises.
5. Only dead or previously euthanized animals are accepted at the KADDL. Horses, livestock, pet, and exotic animals, and animals that pose a safety risk will not be euthanized by KADDL personnel. The owner must make private arrangements for euthanasia of their animal.
6. The KADDL is required by state law to report certain infectious diseases.
7. KADDL is capable of testing appropriate samples for a limited number of common poisonous substances. When testing for poisoning is requested, an additional non-refundable \$25 fee is assessed for collection of samples for those tests conducted at KADDL or another laboratory. Testing is conducted at the discretion of the pathologist on the basis of necropsy findings. The owner/agent will be consulted prior to sample testing at an outside laboratory and is responsible for all sample testing and shipment costs.
8. Submitting samples, specimens, or animals to KADDL implies agreement to policies of the State of Tennessee and KADDL and transfers ownership of samples and property to KADDL upon receipt unless otherwise determined by written agreement prior to submission.

I, the undersigned, do hereby certify that I am the owner, or duly authorized agent for the owner, of the animal(s) submitted to the Kord Animal Disease Diagnostic Laboratory for postmortem examination, have read and do understand the information presented above. I do also certify that the said animal, to the best of my knowledge, has not been exposed to rabies.

Signed _____ Date _____
Owner of Animal or His/Her Agent

INDEX

- Abortion Screen, 11
- Abortions, 8
- Acid Fast Stain, 11
- Actinomyces, 11
- Adenovirus
 - Virology
 - Canine, 37
- Aerobic Specimens
 - Collection and Preparation, 9
 - Culture, 11
- Aflatoxin, 32
- Anaerobic Specimens
 - Collection and Preparation, 9
 - Culture, 11
- Anaplasmosis, 29
- Antibiotic Sensitivity, 11
- Anticoagulant, 32
- Arsenic, 32
- Avian Influenza, 24
- Bacteriology**, 9
- Blastomycosis, 24
- Blood Culture, 11
- Bluetongue, 29
- Bovine Leukosis (BLV), 29
- Bovine Respiratory Syncytial Virus (BRSV)
 - Serology, 29
 - Virology, 38
- Bovine Viral Diarrhea (BVD)
 - Serology, 29
 - Virology, 38
- Bovine Viral Diarrhea Persistent Infection (BVDPI)
 - Serology, 29, 30
- Brodifacoum, 32
- Bromadiolone, 32
- Brucella abortus Culture, 11
- Brucella canis
 - Culture, 11
 - Serology, 24
- BUN, 32
- Calcium, 32
- Calicivirus
 - Serology
 - Canine, 24
 - Virology
 - Canine, 37
 - Feline, 37
- Campylobacteriosis, 11
- Candida, 11
- Caprine Arthritis-Encephalitis (CAE)
 - Serology, 30
- Carbamate, 33
- Chlamydia
 - Avian, 24
 - Ruminant, 30
 - Serology, 26
- Clinical Pathology**, 17
- Clostridium, 30
- Coggins (See Equine Infectious Anemia), 26
- Copper, 33
- Coronavirus
 - Serology (FA)
 - Ruminant, 30
- Cryptosporidia, 11
- Cyanide, 33
- Cytology, 17
- Dermatomycosis, 11
- Dermatophilosis, 11
- Diarrhea/enteritis, 11
- Dicumarol, 33
- Difenacoum, 33
- Distemper Virus
 - Serology, 25
- Drug Screen, 33, 35
- E.coli Pilus
 - Porcine, 28
 - Ruminant, 30
- EAR NOTCH**, 22
- Ehrlichia canis, 25
- Epizootic Hemorrhagic Disease, 30
- Equine Infectious Anemia (EIA), 26
- Equine Rhinopneumonitis Virus (ERV)
 - Serology, 26
 - Virology, 37
- Ethylene glycol, 33
- Fees**, 4
- Feline Calicivirus
 - Serology, 26
- Feline Herpes virus
 - Serology, 27
- Feline Immunodeficiency Virus (FIV)
 - Serology, 27
- Feline Infectious Peritonitis (FIP)
 - Serology, 27
- Feline Leukemia, 27
- Fumonisin, 33
- Gossypol, 33
- Herpesvirus
 - Serology
 - Canine, 25
 - Ruminant, 30
 - Serology (ELISA)
 - Porcine, 28
 - Virology
 - Canine, 37
 - Equine, 37
 - Feline, 37
 - Porcine, 38
 - Ruminant, 38
- Histopathology**, 16
- Histoplasmosis, 25
- Infectious Bovine Rhinotracheitis (IBR)
 - Serology, 30
 - Virology, 38
- Infectious Canine Hepatitis, 25
- Iron, 33
- Johne's
 - Culture, 11, 12
 - Serology, 30
- Lead, 34
- Leptospirosis
 - Porcine, 28
 - Ruminant, 31
- Listeria
 - Culture, 11
 - Serology, 31
- Lyme Disease
 - Serology, 25
- Magnesium, 34
- Metal Screen - ICP, 34
- Milk Specimen
 - Collection, 9
 - Culture, 11

Monensin, 34
 Moraxella bovis, 12
Mycology, 9
 Mycoplasma
 Culture, 12
 Mycoplasma gallisepticum, 24
 Mycoplasma synoviae, 24
 Necropsy, 9, 15
 Neospora caninum
 Serology
 Canine, 25
 Ruminant, 31
 Nitrate, 34
 Ochratoxin, 34
 Organochlorines, 34
 Organophosphates, 34
 Ovine Progressive Pneumonia (OPP)
 Serology, 31
 Panleukopenia
 Serology, 27
 Virology, 37
 Paratuberculosis
 Culture, 11, 12
 Serology, 30
 Parvovirus
 Serology
 Canine, 26
 Porcine, 28
 Virology
 Canine, 37
 Porcine, 38
 Pesticide Screen, 33
 pH, 34
 Pinkeye, 12
 Pneumonia, 12
 Porcine Respiratory and Reproductive Syndrome (PRRS)
 Serology, 28
 Virology, 38
 Postal requirements, 6
 Potassium, 34
 Potomac Horse Fever, 26
 Poxvirus, 38
 Protein, Total, 34
 Pseudorabies
 Serology, 28
 Virology, 38
 Rabies, 36
 Rabies, 39
Reportable Diseases, 41
 Reporting Results, 5
 Rocky Mountain Spotted Fever (RMSF)
 Serology, 26
 Rotavirus
 Serology (ELISA)
 Porcine, 28
 Ruminant, 31
 Salmonella, 12
 Selenium, 34
Serology, 20
 Shipment, 16
 Sodium, 35
 Streptococcus equi, 12
 Strychnine, 35
 Submission Form, 16
 Swab submissions, 9
 Swine Influenza
 Serology, 28
 T-2, 35
Toxicology, 32
 Toxoplasmosis
 Serology, 27
 Transmissible Gastroenteritis (TGE), 29
 Trichomonas, 12
TRICHOMONAS, 12
 Urea, 35
Virology, 36
 Vomitoxin, 35
 Warfarin, 35
 West Nile Virus
 Serology, 26
 Zearalenone, 35
 Zinc, 35