The Indiana Animal Disease Diagnostic Laboratory at Purdue University

West Lafayette, IN

Heeke Animal Disease Diagnostic Laboratory

Dubois County, IN

The Indiana Animal Disease Diagnostic Laboratory at Purdue University (ADDL) serves the people of Indiana by diagnosing disease in livestock, poultry, companion animals, and wildlife, as well as providing blood testing of animals for federal disease programs.

ADDL is under the joint jurisdiction of the Indiana Board of Animal Health and the Purdue University Board of Trustees. It was established at Purdue University in 1945. The original ADDL building at Purdue-West Lafayette was one of the first buildings in the United States completely dedicated to veterinary diagnostic activities.

ADDL's resources enable it to successfully meet its objective: to provide accurate and prompt diagnostic service to veterinary practitioners, animal producers, companion animal owners, wildlife conservationists, animal researchers, and state/federal regulatory officials.

To ensure the quality of our services, the laboratories voluntarily undergo accreditation evaluation and designation. The Purdue laboratories are currently designated full accreditation for all species through 2009 by the American Association of Veterinary Laboratory Diagnosticians. Four senior staff are diplomates of the American College of Veterinary Pathologists; one is a diplomate of the European College of Veterinary Pathologists; two are diplomates of the American College of Poultry Veterinarians; one is a member of the American Society of Microbiologists; one is a diplomate of the American Board of Veterinary Toxicology.

Various sections of the ADDL participate in proficiency tests conducted by the National Veterinary Services Laboratory and other quality control evaluations. Because of ADDL's nationally/internationally recognized facilities and staff, Indiana practitioners and animal producers can be assured of quality service.

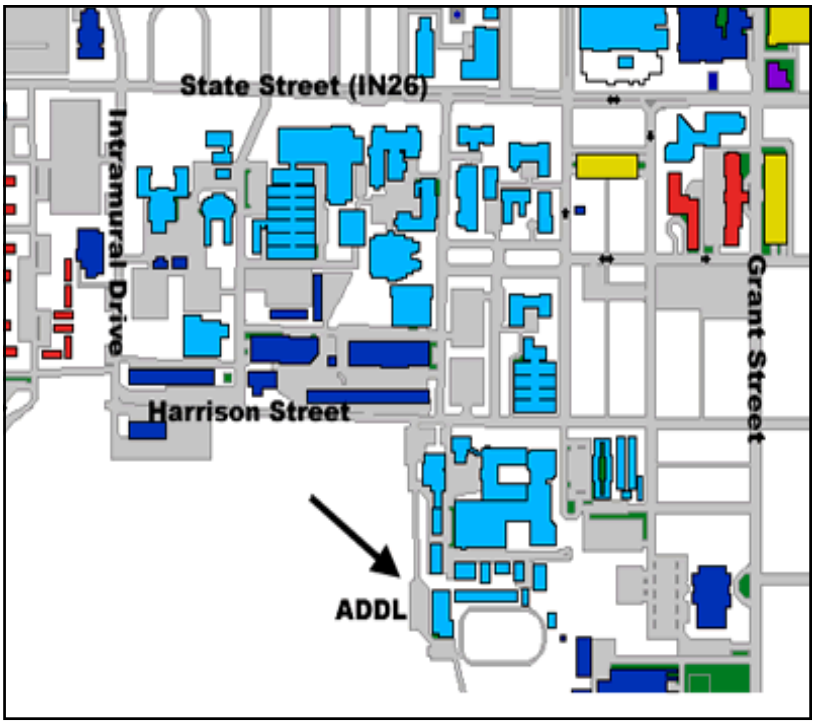
Hours

The Purdue Animal Disease Diagnostic Laboratory (West Lafayette, IN) and Heeke Animal Disease Diagnostic Laboratory (Dubois, IN) are open from 8:00am until 5:00pm Monday through Friday.

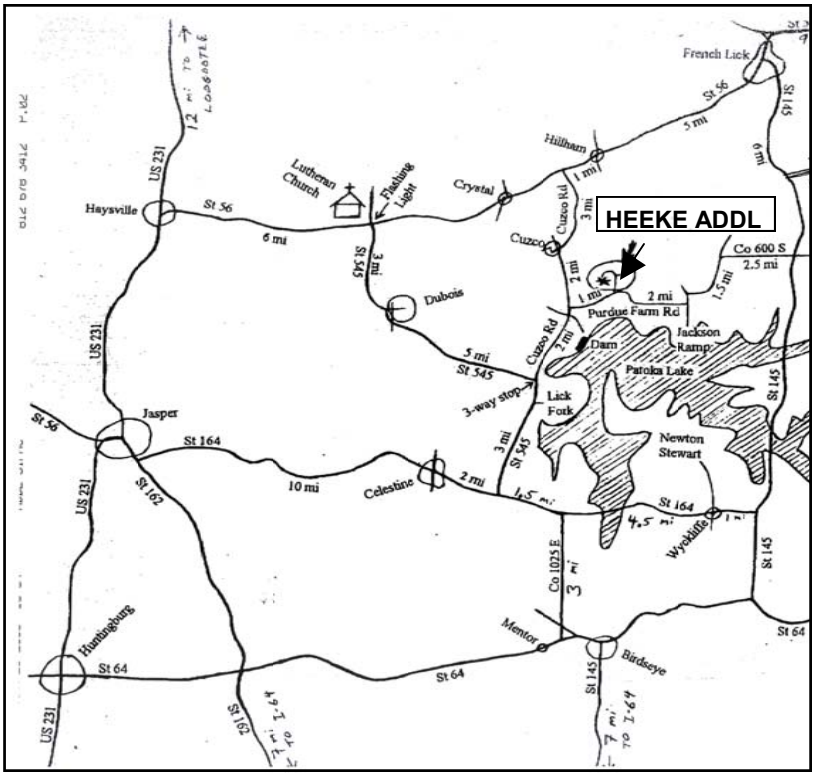
The laboratories are closed on those holidays observed by Purdue University.

Faculty and staff are available after hours for emergencies only.

Call 765-494-7440 and follow the instructions on the answering machine.



Purdue ADDL is located on the south end of Purdue University's West Lafayette campus at the intersection of Harrison and South University Streets.



Heeke ADDL is located at the Southern Indiana Purdue Agricultural Center near Dubois, Indiana, close to the Lake Patoka Recreation Area.

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Contents are subject to change without notice.

For the most current information on new tests, fees, and testing guidelines, please visit our web page at ***www.addl.purdue.edu***

ADDL Test Results are Available on the Internet

To set up an Internet account, please do one of the following:

1) Call ADDL at 765-494-7440 and ask to speak to the Computer Systems Manager.

or

2) -Log on to our website at www.addl.purdue.edu.
-Click on the Online Reports tab at the top of the page.
-Click on "Request Info" on the left navigation bar and follow the instructions.

Supplies Provided by ADDL

The following supplies can be sent to you at your request.

Histopathology mailing jars (neoplasms) **Includes formalin-filled container, outside mailing container, pre-paid address label**	**$25/12**
Whirl-Pak bags (lots of 50)	**$5.00/lot of 50**
Blood tube boxes (24s) **To be used only for blood samples sent to Purdue ADDL**	**N/C**
Blood tube boxes (100s) **To be used only for blood samples sent to Purdue ADDL**	**N/C**
Accession Form 1 **Downloadable versions of the accession form are also available on the ADDL web page (www.addl.purdue.edu)**	**N/C**
Accession Form 2—Neoplasms **Downloadable versions of the Neoplasm accession form are also available on the ADDL web page www.addl.purdue.edu**	**N/C**
Serology "buckslip"	**N/C**

General Guidelines

To ensure prompt service and accurate results, please follow these general guidelines when submitting cases to ADDL.

1. Be sure all forms are complete and legible. Include owner's name and address, and veterinarian's name, address and license number. Downloadable ADDL accession forms are available on the ADDL website at <www.addl.purdue.edu.>

2. If specimens of the same organ are to be sent to two or more labs within ADDL (e.g.. virology, bacteriology, toxicology) they must be packaged and labeled separately (one bag for each lab requested).

3. When calling for results, refer to case by accession number. If accession number is not available, identify your owner and veterinarian exactly as written on the test chart or the diagnostic accession form.

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4. Please DO NOT submit materials for diagnostic procedures on the Brucellosis form (VS 4-33). Use ADDL Diagnostic Form 1 or the ADDL Request for Neoplasm Report.

5. Case information can only be released to the veterinarian or owner specified on the submission form. For release to a third party, written permission is needed.

6. Specimens submitted for examination may undergo additional diagnostic testing for state or federal disease surveillance programs, particularly involving foreign animal diseases.

7. Fees are subject to change without notice. Please visit our web page <www.addl.purdue.edu> for the most current information on fees, new tests, forms, testing requirements.

Information to Include in Case History

1. Owner's name and address
2. Species, breed, sex, age, and weight of affected animals
3. Number of animals in herd or flock
4. Morbidity and mortality
5. Complete description of clinical signs
6. Time the animal was last observed prior to death and condition at that time
7. Detailed description of your necropsy findings if applicable
8. Treatment, time of treatment, and animal response
9. Detailed description of management practices

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Quick Reference Guide

Sample Submissions

The following are suggestions for etiologic agents to consider when determining diagnostic procedures to request related to specific disease syndromes. While not comprehensive, it will offer guidelines for choosing appropriate diagnostic samples to submit for specific tests.

It is advisable to submit freshly dead or symptomatic, untreated whole animal carcasses for a complete post mortem examination. In addition to the chilled fresh samples listed, it is usually advisable to submit formalin-fixed tissues for histopathologic examination. For questions concerning any diagnostic procedure, call the Purdue ADDL located on Purdue's campus in West Lafayette at (765) 494-7440 or the Heeke ADDL located in Dubois, In at (812) 678-3401.

Key

FA……...Fluorescent Antibody
VI……...Virus isolation
ELISA.....Enzyme Linked Immunosorbent Assay
IFA……..Indirect fluorescent antibody
AGID…..Agar gel immunodiffusion
VN…..…...Virus neutralization
CF…….....Complement fixation
PCR.....….Polymerase chain reaction
EM…….....Electron microscopy

Abortions

Check our website <www.addl.purdue.edu>for the latest information on abortion panels.

For abortions, irrespective of species, the following tissues/specimens should be submitted.

Tissue or Specimen	Fresh, Chilled (not frozen)	Fixed (10% formalin)
Placenta	√	√
Fetal lung, liver, kidney, spleen, brain, heart, lymph node	√	√
Fetal fluid (thoracic or peritoneal)	√	
Serum (from dam)	√	
Fetal stomach or abomasal fluid	√	

Listed in the following sections are preferred specimens for specific infectious abortifacients. If multiple procedures are available for a test, please specify which procedure you are requesting.

Abortions

Agent	Preferred Specimen	Procedure
Abortions-Bovine bacterial		
Brucella abortus	-Fetal fluid, serum from dam -Placenta, fetal lung, kidney, abomasal fluid, spleen	Serology Culture
Campylobacter	-Fetal kidney/spleen	Culture
Leptospira	-Fetal tissues -Serum (from dam)	PCR, Serology
Abortions—bovine parasitic		
Neospora	-Serum (from dam) -Any tissue with lesions	Serology PCR
Abortions—Bovine viral		
Bluetongue virus (BT)	Serum	Serology
BVDV (Bovine viral diarrhea virus)	-Fetal thoracic/abdominal fluid -Placenta, fetal lung, spleen, kidney, brain, intestine, lymph node, serum -Serum from dam (acute and convalescent)	Serology (VN) FA, VI, PCR, IHC Serology
IBR (Infectious Bovine Rhinotracheitis virus)	-Fetal thoracic/abdominal fluid -Placenta, fetal lung, spleen, kidney, brain, intestine, lymph node -Serum from dam (acute and convalescent)	Serology (VN) FA, VI Serology
PI3 (Parainfluenza virus)	-Fetal thoracic/abdominal fluid -Placenta, fetal lung, spleen, kidney, brain, intestine, lymph node -Serum from dam (acute and convalescent)	Serology (VN) FA, VI Serology
Abortions—Equine bacterial		
Brucella ovis	-Fetal thoracic/peritoneal fluid -Placenta, fetal lung, liver, stomach fluid -Serum	Serology Culture Serology
Campylobacter	Fetal liver, kidney	Culture
Chlamydia	Placenta, liver	PCR
Coxiella	Placenta, lung, liver, lymph node, spleen	Culture (sent to NVSL), PCR at ADDL
Leptospira	-Urine, liver, kidney, placenta -Serum	PCR Serology
Other bacteria	Placenta, fetal organs	Culture
Streptococcus zooepidemicus	Placenta, fetal lung, liver, kidney	Culture
Abortions—Equine viral		
Equine herpesvirus 1	-Placenta, fetal lung, liver, adrenals, spleen -Serum -Nasal swab (Dacron), tracheal wash, CSF, placenta, lung, liver	FA, VI Serology PCR
Equine viral arteritis	-Thoracic or peritoneal fluids, serum from dam -Placenta, fetal lung, spleen, bronchial lymph node	Serology VI
Abortions—Equine parasitic		
Toxoplasma gondii	-Fetal fluid, serum from dam	Serology

Agent	Preferred Specimen	Procedure
Abortions—Ovine/Caprine bacterial		
Brucella ovis	-Fetal thoracic/peritoneal fluids, serum -Placenta, fetal lung, liver, kidney, and abomasal fluid	Serology Culture
Campylobacter	Fetal liver, spleen and kidney	Culture
Chlamydia	Placenta, liver, kidney	PCR
Coxiella	Placenta, lung, liver, lymph node, spleen	9
Leptospira	Urine, liver, kidney, placenta	PCR
Abortions-Ovine/Caprine viral		
Bluetongue virus (BT)	Serum	Serology
Border disease virus (BDV)	-Fetal thoracic/peritoneal fluids -Placenta, fetal lung, thyroid, brain, skin, serum	Serology FA, VI, PCR
Abortions-Ovine/Caprine parasitic		
Toxoplasma gondii	-Fetal fluid, serum from dam -Fetal tissues, placenta	Serology PCR
Abortions—Porcine bacterial		
Brucella	-Placenta, fetal organs -Serum from dam	Culture Serology
Leptospira	-Urine, liver, kidney -Serum	PCR Serology
Other bacteria (e.g. *Actinobacillus)*	Fetal organs	Culture
Abortions—Porcine viral		
Parvovirus	-Placenta, fetal lung, kidney -Serum from dam	FA, VI Serology
PRRSV (Porcine Reproductive and Respiratory Syndrome virus)	-Serum from sow, fetal thoracic fluids -Serum, spleen, tonsil, fetal fluids	Serology VI, PCR, IHC
Pseudorabies virus	-Placenta, fetal tonsil, lung, spleen, brain -Serum	FA, VI Serology
Porcine circovirus	Fetal thoracic/peritoneal fluids, lung, heart	VI, FA, IHC

Diarrhea

Agent	Preferred Specimen	Procedure
Diarrhea—Bovine/Ovine/ Caprine bacterial		
Johne's Disease (*Mycobacterium paratuberculosis*)	-Feces, large intestine, regional lymph node, ileum -Serum	Culture, PCR Serology
Salmonella	Colon, ileum, lymph node, spleen, lung, liver, feces	Culture, PCR
E. coli	Ileum, colon, feces	Culture +/- PCR typing
Clostridium perfringens	Small intestine, intestinal contents (within 6 hours of death or snap frozen)	Anaerobic culture +/- PCR typing
Diarrhea—Bovine/Ovine/ Caprine viral		
Coronavirus	-Spiral colon, ileum, feces -Feces -Feces, intestinal contents	FA +/- VI EM RT-PCR
Rotavirus	-Upper, middle, lower small intestine, 6" segments tied off (DO NOT OPEN GUT) -Feces	FA, VI EM, AgcELISA
BVD (Bovine viral diarrhea)	-Spleen, mesenteric lymph node, rumen, ileum -Buffy coat (Submit EDTA blood for acutely infected animals and persistently infected calves -Serum -Lymph node, spleen, blood, serum	FA, VI VI, AgcELISA Serology PCR
Diarrhea—Bovine/Ovine/ Caprine parasitic		
Cryptosporidia	-Feces -Intestine fixed in formalin	Parasitology Histopathologic exam
Coccidia	-Feces -Intestine and colon fixed in formalin	Flotation Histopathologic exam
Diarrhea-Canine/Feline bacterial		
E. coli	Feces, intestine	Culture
Salmonella	Feces, intestine	Culture, PCR
Campylobacter	Feces, intestine	Culture
Clostridium	Feces, intestine	Culture +/- PCR typing

Agent	Preferred Specimen	Procedure
Diarrhea—Canine/Feline viral		
Canine coronavirus	- Feces - Small intestine - Serum	VI, EM, PCR FA, VI Serology
Canine parvovirus	- Feces - Small intestine, spleen, tongue - Serum	VI, EM, PCR FA, VI Serology
Canine rotavirus (Group A)	- Feces - Small intestine	EM FA
Feline enteric coronavirus	- Small intestine, segments of distal duodenum, ileum, jejunum - Serum	FA Serology
Feline panleukopenia	- Spleen, lung, distal jejunum, ileum, fetal tissues - Serum	VI, FA Serology
Feline rotavirus	- Feces - Intestine	VI, EM FA, VI
Diarrhea—Equine bacterial		
Salmonella	Feces, ileum, lymph node, spleen, lung, liver	Culture, PCR
Clostridium difficile	Feces, ileum, colon	Anaerobic culture +/- PCR typing
Clostridium perfringens	Feces, small intestine, intestinal contents	Anaerobic culture +/- PCR typing
Rhodococcus equi	Ileum, colon	Culture
Diarrhea—Equine viral		
Rotavirus	-Feces -Small intestine	EM FA
Diarrhea—Equine parasitic		
Strongyles/Coccidia	-Feces -Ileum, colon fixed in formalin	Fecal flotation Histopathologic exam
Diarrhea—Porcine bacterial		
Salmonella	Colon, ileum, lymph node, spleen, lung, liver, feces	Culture, PCR
E. coli	Ileum, duodenum, colon, feces	Culture +/- PCR typing
Brachyspira hyodysenteriae and/or Brachyspira pilosicoli	Colon	Culture, PCR
Clostridium perfringens	Ileum contents (within 6 hours of death or frozen), small intestine, large intestine	Anaerobic culture +/- PCR typing
Diarrhea—Porcine parasitic		
Cryptosporidia	Feces, small intestine	Stain
Coccidia	-Feces -Intestine fixed in formalin	Fecal flotation Histopathologic exam
Diarrhea—Porcine viral		
TGE (Transmissible gastroenteritis)	-Feces -Ileum, small intestine, colon -Serum	EM FA Serology
Rotavirus	-Feces -Ileum, small intestine, colon -Serum	EM FA Serology

Respiratory

Agent	Preferred Specimen	Procedure
Respiratory—Bovine bacterial		
Pasteurella *Hemophilus* *Mycoplasma* *Actinomyces* *Salmonella*	Swabs of nasal and ocular secretions, affected lung, bronchial lymph node, tracheal wash	Culture
Respiratory—Bovine viral		
BVDV (Bovine Viral Diarrhea virus)	- Buffy coat (EDTA blood) - Lung, intestine, spleen, fetal organs - Serum (Acute and convalescent)	VI, PCR FA, VI, PCR Serology, PCR
IBR (Infectious Bovine Rhinotracheitis virus)	- Nasal secretions, trachea, lung, spleen - Fetal lung, spleen, kidney, liver - Serum (acute and convalescent)	FA, VI FA, VI Serology
PI3 (Parainfluenza virus)	- Nasal secretions, trachea, lung, spleen - Fetal lung, spleen, kidney, liver - Serum (acute and convalescent)	FA, VI FA, VI Serology
BRSV (Bovine Respiratory Syncytial virus)	- Affected lung portions, trachea - Serum (acute and convalescent)	FA Serology
Respiratory Canine/Feline Bacterial		
Pasteurella	Lung, bronchial lymph node, nasal swab, transtracheal wash	Culture
Bordetella	Lung, bronchial lymph node, nasal swab, transtracheal wash	Culture
Respiratory Canine/Feline Viral		
Canine adenovirus	Nasal secretions, liver, kidney	VI
Canine distemper	Conjunctival smears, brain, lung, spleen	FA, VI
Canine influenza	Lung, nasal swabs (Dacron), trachea	PCR, VI
Canine parainfluenza	- Nasal/throat swab, lung - Serum	VI Serology
Feline calicivirus	Lung, gingival/nasal swabs	VI
Feline rhinotracheitis (Herpesvirus)	- Nasal/ocular pharyngeal swabs lung - Serum	VI, FA
Feline infectious peritonitis (coronavirus)	- Thoracic fluid, peritoneal fluid, lung, liver, kidney - Serum	FA, VI Serology
Respiratory—Equine bacterial		
Streptococcus equi	Lung, lymph node, nasal swabs, transtracheal wash	Culture
Rhodococcus equi	Lung, bronchial lymph node, tracheal wash	Culture
Streptococcus zooepidemicus	Lung, bronchial lymph node, tracheal wash	Culture

Agent	Preferred Specimen	Procedure
Respiratory—Equine viral		
Equine rhinopneumonitis	- Trachea, lung, nasal/gingival swab - Fetal lung, liver, kidney - Serum	VI FA, VI Serology
Equine herpesvirus 4	Throat/lung swabs	VI
Equine influenza	Trachea, lung, nasal swabs	FA, VI
Equine viral arteritis	- Nasal swab, lung, bronchial lymph node - Serum	VI Serology
Respiratory—Ovine/Caprine bacterial		
Pasteurella *Hemophilus* *Mycoplasma* *Actinomyces*	Swabs of nasal and ocular secretions, affected lung, bronchial lymph node, tracheal wash	Culture
Respiratory—Ovine/Caprine viral		
RSV (Respiratory Syncytial Virus)	Affected lung, trachea	FA, VI, Serology
PI3 (Parainfluenza virus)	- Affected lung - Serum	FA, VI Serology
OPP (Ovine Progressive Pneumonia)	Serum	Serology
Caprine Arthritis Encephalitis	Serum	Serology
Respiratory—Ovine/Caprine parasitic		
Ascarids	Feces	Flotation
Lungworms	- Feces - Lung (fixed in formalin)	Flotation Histopathologic exam
Respiratory—Porcine bacterial		
Bordetella *Actinobacillus* *Salmonella* *Streptococcus suis*	Lung, bronchial lymph node, nasal swab, tracheal wash	Culture
Mycoplasma	Lung, tracheal wash	FA, Culture
Respiratory—Porcine viral		
Pseudorabies virus	Brain, spleen, tonsil, lung	FA, VI
Swine influenza virus	- Tonsil, lung, nasal swab (Dacron) - Serum	FA, VI, PCR Serology
PRCV (Porcine Respiratory Corona virus)	Lung, nasal swabs (Dacron)	PCR, VI
PRRSV (Porcine Respiratory and Reproductive Syndrome virus)	- Serum (clear), lung, spleen, tonsil, lymph node - Serum	VI, PCR Serology
Porcine circovirus	- Lung, lymph node, tonsil, kidney - Serum	PCR, FA, VI, IHC Serology

Aquaculture

The following guidelines are provided for submission of fish**.** ADDL accepts specimens from all species of fish.

1. The best sample for submission is an acutely affected, live fish exhibiting clinical signs or having gross lesions of disease. Three to five adult fish or 20-25 fingerlings are recommended for fish kill cases.

2. Sixty fish per lot are required for submission for fish health inspection. Fish submitted for such purpose should be collected under the supervision of a fish pathologist, fish health specialist, veterinarian, or fish biologist.

3. The best method for transporting fish to the ADDL is hand delivery, with fish in a clean bucket, or plastic or styrofoam cooler in water from the environment from which the fish originated. If transportation time is greater than 1-2 hours, it is recommended that a small battery-operated aerator be used for supplemental oxygen.

4. For overnight shipment, place fish in a large, thick, transparent plastic bag filled approximately 1/3 full with water. An "air cap" of oxygen should be present immediately above the water surface, occupying at least 1/3-1/2 of the plastic bag. The bag should be securely tied and placed inside another bag to prevent leakage. This bag should be placed in a thick, wax-coated cardboard box for shipping.

5. It is good practice to conduct the standard water quality tests (including the dissolved oxygen concentration) on site. The water sample can be submitted along with the fish submission for aquatic toxicology examination at ADDL. The water sample should be submitted in a clean, one quart glass jar with a screw top lid (e.g.. canning jar) with a layer of aluminum foil placed between the water sample and the lid. Water samples shipped in this manner are satisfactory for pesticide or herbicide analysis.

6. A complete history should be included with each submission. Include husbandry, water quality problems, stocking densities, size of the tank/pond/lake, approximate mortality and morbidity, algal bloom history, origin of the fish, and date and time of onset of current problem.

7. Aquatic diagnostics include aquatic necropsy, histopathology, bacteriology, virology, and toxicology.

8. Aquatic specimens submitted for bacteriology, virology, and/or toxicology examination will be processed by the Bacteriology and Mycology Section, the Virology Section, and Toxicology Section in the ADDL, respectively, and should comply with submission requirements specified by the appropriate section as indicated in this manual.

9. For tissues submitted for aquatic histopathology:

    - Tissues should not be more than 0.5 cm thick and should be submitted in wide mouth jars containing 10% buffered formalin. The volume of tissue specimens to that of 10% formalin in a jar should be a 1:10 ratio.
    - Suggested tissue specimens include skull, vertebrae, brain, gill, heart, liver, anterior/posterior kidney, swim bladder, stomach, intestine, skin, fins, as well as specific lesions in any organ/ tissue observed at gross examination.

10. Several special procedures are available for the detection of aquatic pathogens in the ADDL, including immunofluorescent antibody assay (IFA) for bacterial kidney disease (*Renibacterium salmoninarum*) and PCR for Whirling Disease (*Myxobolus cerebralis*). Other special procedures may be arranged on request by contacting ADDL at 765-494-7440.

Avian

General Information

1. The Avian Section of the ADDL accepts specimens from all species of birds, including poultry, game birds, caged birds, wild birds, and zoo birds.
2. The Avian Section performs a number of procedures for avian submissions, but also utilizes the services of other sections to avoid duplication of procedures whenever possible.
3. Avian specimens submitted only for microbiological culture or toxicological analysis should comply with submission requirements specified by the appropriate section as shown in this manual.
3. If help is needed in determining the best type of specimen to submit, contact the ADDL Avian Section at 765-496-3347 or 765-494-7454. In southern Indiana, call Heeke ADDL at 812-678-3401.

Avian Necropsy

1. For suspected flock problems, submit six (6) clinically affected adult birds, or up to 25 affected chicks. Cull birds are inappropriate specimens. Place birds in a suitable container that provides adequate space and ventilation.
2. For problems involving excessive mortality, submit six (6) fresh dead adult birds or up to 25 fresh dead chicks. Do not submit decomposed birds.
3. For individual bird submissions, wet the carcass with soapy water, wrap in wet paper, and place in plastic bag. Keep the sample refrigerated until submitted. Avoid freezing when possible.
4. Only those dead birds that cannot be submitted to ADDL within 48 hours of death should be frozen.

Avian Histopathology

1. Samples of tissues, not more than 0.5 cm thick, should be submitted in widemouth jars containing 10% neutral-buffered formalin. The volume of tissue specimens to that of 10% formalin in the container should be in 1:10 ratio. Suggested tissues include brain, lung, trachea, liver, kidney, spleen, intestines (including duodenum, jejunum and ileum), bursa, and heart, as well as specific lesions in any organ observed at gross necropsy.
2. Skin biopsies submitted for examination for Beak and Feather disease should contain at least 2-3 feather follicles and/or newly emerged dystrophic feathers.

Avian Bacteriology

1. A portion of the following tissues should be refrigerated for bacteriology examination. Suggested samples include brain, trachea, sinus/choanal swab, lung, liver, kidney, spleen, small intestine, cecum, and cloacal swab.
2. Avian specimens submitted for bacteriology examination will be processed by the ADDL Bacteriology and Mycology section.

Avian Virology

1. A portion of the following tissues should be refrigerated for virology examination: brain, lung, trachea, sinus/choanal swab, liver, spleen, and cecal tonsil.
2. Egg inoculation will be performed for avian specimens submitted for virology examination.

Avian Serology

1. A variety of serological procedures for detection of antibodies against the most common avian pathogens are available through the Avian Section of ADDL. Routinely performed procedures include:

:

Serum Plate Agglutination
Mycoplasma gallisepticum
Mycoplasma synoviae
Mycoplasma meleagridis
Salmonella pullorum/gallinarum
Salmonella enteritidis
Salmonella typhimurium

Tube Agglutination
Salmonella enteritidis
Salmonella pullorum/gallinarum
Salmonella typhimurium

Agar Gel Precipitation
Avian Adenovirus
Avian Influenza Virus
Infectious Bursal Disease Virus

Hemagluttination inhibition
Mycoplasma gallisepticum
Mycoplasma synoviae
Mycoplasma meleagridis
Newcastle Disease Virus
Pigeon paramyxovirus 1
Paramyxovirus 2
Paramyxovirus 3

ELISA
Avian Influenza Virus
Avian Encephalomyelitis Virus
Bordetella avium
Hemorrhagic Enteritis Virus
Infectious Bronchitis Virus
Infectious Bursal Disease
Mycoplasma gallisepticum
Mycoplasma synoviae
Reovirus

2. Serum samples submitted for testing should be in properly labeled tubes.

3. Blood samples should be taken in red top tubes and the tube immediately placed on its side to allow blood to clot. Serum should be separated from clotted blood prior to submission for testing. Do not allow whole blood to freeze or be exposed to direct sunlight or high temperature prior to decanting serum.

4. Approximately 0.5-1.0 ml of serum is required for testing purposes.

5. Flock serology should be based on a sample size of 20-30 serum samples. Regulatory or NPIP testing may require larger numbers of samples. Check with the Avian Section prior to submission.

6. Serological diagnosis of disease is only possible when a comparison of antibody levels is made between those measured at the time of clinical disease (acute samples) and those measured approximately 4 weeks later (convalescent samples). Both acute and convalescent serum samples should be submitted in order to derive the most meaningful diagnosis from serologic testing.

7. Other serological procedures may be arranged by special request by contacting the Avian Section at 765-494-7454 or 765-496-3347.

Avian Antigen Immunoassay

Several avian pathogens can be detected by rapid immunocapture procedures. Swabs, exudates, or tissue specimens from sinus, choana, trachea, liver, spleen, intestine, or cloaca may be submitted for these procedures.

Rapid Immunocapture Assay
Chlamydophila

Rapid Immunomigration Test
Avian Influenza Virus (Flu Detect)

Avian Toxicology

1. Suggested tissues or specimens include brain, liver, crop/proventriculus/gizzard content, feed, and/or water.

2. Tissues or specimens submitted for toxicologic examination will be processed by the ADDL Toxicology Section.

Avian Molecular Diagnostics

1. PCR or RT-PCR procedures are available for swabs, exudates, or tissue specimens from sinus, choana, trachea, lung, liver, spleen, intestine, or cloaca for the detection of *Chlamydophila psittaci, Mycoplasma gallisepticum, Mycoplasma synoviae*, Newcastle Disease Virus, and Avian Influenza Virus

2. Other PCR procedures may be arranged by special request by contacting the Avian Section at 765-494-7454 or 765-496-3347.

Bacteriology

The Microbiology Service of the ADDL provides examinations for a wide variety of diseases affecting the animal population of Indiana. Some of the most common are listed in the following pages. In addition to those services listed, the following are available.

1. **Antibiotic susceptibility tests of recovered isolates**. Please indicate on the accession form that you are requesting susceptibility testing.

2. **Identification of bacterial isolates**. PLEASE SEND ONLY PURE CULTURES FOR IDENTIFICATION. If mixed cultures are received, only the most predominant colony type will be identified unless sender specifies otherwise.

3. **Serogrouping of *Salmonella***. *Salmonella* isolates will be checked for serogroups at the ADDL and the isolate forwarded to NVSL for definitive typing. A final report from NVSL may take four weeks or longer.

4. **Return or forwarding of isolates recovered from clinical material**. If you would like this service, the request to save the isolate must be made at the time of submission. Be specific as to which isolate you would like saved. ADDL will forward isolates to reference laboratories (Submitter will be asked to complete paperwork for the reference laboratory, and fax it to ADDL prior to shipment of isolate; postage charges will be incurred.)

5. All *Streptococcus suis* isolates are checked against antisera 1-34 unless requested otherwise.

6. *Actinobacillus pleuropneumoniae* isolates are checked against antisera 1-12.

7. Some tests that are not performed at Purdue ADDL are available at other laboratories.
   --Cultural examination for *Trichomonas* is done at the University of Wisconsin. Purdue will accept the commercial "one pouch" system and provide interpretation.

   --Samples suspected of Contagious Equine Metritis (CEM) will be forwarded to NVSL for processing. The Breathitt Veterinary Center, Hopkinsville, Kentucky, will accept submissions for cultural examination for CEM. Specimens must be submitted in Aimes Transport Medium. Call the Breathitt Center at 270-886-3959 prior to submitting samples.

8. Specimens suspected of harboring anaerobic bacteria must be shipped in an anaerobic container.

Collection and Care of Milk Samples

Valid bacteriology laboratory results for mastitis control require that contaminating bacteria be kept to an absolute minimum. This requirement is relatively difficult to achieve since 1) the cow's environment is rich in bacterial contaminants and 2) milk is an excellent culture medium. Bacterial contaminants can be kept to a minimum by following these precautions.

1. Collect the milk aseptically.

   - Brush dirt off the cow's flanks.
   - Scrub the udder with a surgical detergent, rinse well, and dry with clean paper towels. The order of washing, rinsing and drying should be the far side first and the near side last.
   - Apply iodine or alcohol to the teat orifice ,and allow to dry.
   - Discard three or four streams of milk into CMT paddle and test. Collect 5-10 mls from quarters with 2+ CMT value (or greater) into a sterilized screw-capped tube tiled to minimize contamination by airborne bacteria. Collect specimens from near quarters first and far quarters last.

2. Tighten the screw cap, label with an indelible marker (cow number and quarter), and immediately place in cooler.

3. Keep the specimens cold until they are delivered to the laboratory. Delivery should be made on the day of collection.

4. If you are planning to submit more than eight milk specimens at one time, please notify the ADDL to schedule the submission so that adequate time is allowed for preparation of the necessary culture media.

5. If samples were frozen, please indicate on the submission form. A larger sample size is necessary for culture to compensate for the loss of a log of organism through freezing and thawing.

Disease	Species	Agent	Preferred Specimen	Comments
Abortion	All	Various	Aborted fetus, placenta, fetal lung, liver, kidney, stomach contents	-Mycotic abortions are best diagnosed by Histopathology.
Abscesses	All	Various	Aspirate from unopened abscess	Collect exudate from non-draining lesion. *Swabs are of limited value.*
Actinobacillosis		*Actinobacillus*	Exudate or lesion	Collect exudate from non-draining lesion.
Actinomycosis		*Actinomyces sp*	Exudate or lesion	Collect exudate from non-draining lesion; ship in an anaerobic container.
Anthrax	Ruminant Equine Porcine Canine	*Bacillus anthracis*	Blood, spleen or lymph node Pharyngeal lymph node or fluid from pharyngeal lesion	Necropsy contraindicated
Arthritis		Various	Fluid from affected joints	Indicate if *Mycoplasma* is requested.
Aspergillosis		*Aspergillus sp.*	Lesion	
Atrophic rhinitis	Porcine	*-Bordetella bronchiseptica* *-Pasteurella multocida*	Snout. Swab of nasal secretion	
Bacillary hemoglobinuria		*Clostridium hemolyticum*	Liver	Ship specimen in anaerobic container.
Bacterial kidney disease	Fish	*Renibacterium salmoninarum*	Kidney	FA for the presence of bacteria is available, as well as culture (takes 8-12 weeks).
Blackleg	Bovine	*Clostridium chauvoei*	Affected muscle	Ship specimen in an anaerobic container. FA exam performed; if positive, culture is not done
Blastomycosis		*Blastomyces dermatitidis*	Lung or bronchial aspirate	Cultural examination may take up to 5 weeks.
Brooder pneumonia	Avian	*Aspergillus fumigatus*	Affected air sacs	Isolation time is variable.
Brucellosis	Bovine Canine	*Brucella* spp. *Brucella canis*	-Milk—4 quarter samples. Supra-mammary lymph nodes, fetal kidney, lung, stomach contents, semen (2ml) - Uncoagulated blood, lymph nodes, bone marrow, semen, urine, vaginal discharge, milk	-Isolation time is variable. -Serologic tests are available at Purdue.
Calf diphtheria	Bovine	*Fusobacterium necrophorum*	Lesion from oral pharyngeal or laryngeal membranes	Ship in an anaerobic container.
Calf scours	Bovine	*E. coli , Clostridium spp.*	Feces or affected intestine	
Campylobacter infection (vibriosis)	Bovine/ Ovine	*Campylobacter*	Vaginal mucosa, fetus, post-parturient exudates	See Quick Reference Guide section for more information on submission of abortion cases
Candidiasis		*Candida albicans*	Lesion	
Caseous lymphadenitis	Caprine/ Ovine	*Corynebacterium pseudotuberculosis*	Affected lymph node	Isolation time is variable.
Cat scratch disease	Feline	*Bartonella*	-Blood in lysis tube (yellow top) -Tissue with lesions	Should arrive at ADDL 24-48 hours after collection. .

Disease	Species	Agent	Specimen	Comments
Chlamydia infections		*Chlamydia spp.*	Affected tissue	PCR is available. See Molecular Diagnostics section.
Chronic respiratory Disease (CRD-chickens)	Avian	*Mycoplasma gallisepticum*	Air sac, trachea, lung, exudate	Isolation time is a minimum of 30 days. PCR is available. See Molecular Diagnostics section.
Coccidiomycosis		*Coccidioides immitis*	Lung	Cultural examination may take up to 5 weeks.
Coryza	Chickens Turkeys	*-Hemophilus paragallinarum* *-Bordetella avium*	Affected trachea Affected trachea	Isolation time is 2-4 days Isolation time is 2-4 days.
Cryptococcosis		*Cryptococcus neoformans*	Cat: Nasal or skin swab Dog: Cerebrospinal fluid (antemortem) Lesion (at necropsy)	Isolation time is a minimum of 30 days.
Dermatomycosis		*Microsporum/ Trichophyton spp.*	Lesion. Broken hairs or any that fluoresce under Woods lamp	Scrape periphery of lesion. Culture may take up to 5 weeks.
Dermatophilosis		*Dermatophilus congolensis*	Lesion	Remove and send entire scab.
Diamond skin disease	Porcine	*Erysipelothrix rhusiopathiae*	Whole animal (necropsy) or affected organs	May be difficult to isolate and differentiate from normal flora of skin
Diarrhea		Various	Feces	Indicate on accession form if any specific agents are suspected (such as Johne's, *Campylobacter, Clostridium*).
Edema disease	Porcine	*E. coli (*hemolytic*)*	Small intestine	Serotyping (PCR) is available at Purdue. See Molecular Diagnostics section.
Enteritis		Various	Affected intestine	Indicate on accession form if any specific agents are suspected.
Enterotoxemia		*Clostridium perfringens*	Small intestine	Specimen must be fresh.and shipped in an anaerobic container. PCR typing is available. See Molecular Diagnostics.
Enzootic pneumonia	Porcine	*Mycoplasma hyopneumoniae*	Lung	FA exam is performed for *M. hyorhinis* and/or *M. hyopneumoniae.*
Epizootic hemorrhagic septicemia	Ovine	*Pasteurella multocida*	Spleen	Isolation time is 2-3 days.
Feline pneumonitis	Feline	*Chlamydia*	Conjunctival swab, nasal swab	PCR is also available. See Molecular Diagnostics section.
Fibrinous polyserositis of fowl (New Duck Disease)	Avian	*Pasteurella anatapestifer*	Pericardial exudate, liver, brain, lung	Found in ducks, chickens, turkeys
Footrot	Bovine/Ovine	*Bacteroides nodosus/ Fusobacterium necrophorum/others*	Interdigital swab or biopsy, foot lesions	Ship specimen in anaerobic container.
Fowl cholera	Avian	*Pasteurella multocida*	Affected tissue	Isolation time is 2-3 days.
Glassers disease (Infectious polyserositis)		*Hemophilus parasuis*	Inflamed serosa	Deliver to laboratory on day collected or freeze
Hemorrhagic bowel syndrome	Porcine	*Campylobacter, Clostridium, Lawsonia, Brachyspira*	Affected intestine	Culture. PCR is available. See Molecular Diagnostics section.
Hemorrhagic enteritis	Porcine	*Salmonella Clostridium perfringens B&C*	Affected intestine, feces	*Clostridium* typing PCR is available on request.
Histoplasmosis		*Histoplasma capsulatum*	Lung or tracheal wash	Cultural exam may take 8 weeks.
Infectious keratoconjunc-tivitis (pinkeye)	Bovine	*Moraxella bovis*	Swab of affected eye	Isolation may be difficult due to presence of normal flora.

Disease	Species	Agent	Specimen	Comments
Ileitis	Porcine/Equine	*Lawsonia intracellularis*	Feces, intestine	PCR
Infectious sinusitis	Turkey	*Mycoplasma gallisepticum*	Air sac or sinus exudate	Isolation time is a minimum of 30 days. PCR available. See Molecular Diagnostics section.
Infectious synovitis	Chicken/ Turkeys	*Mycoplasma synoviae*	Air sac, liver, spleen, synovial exudate	Isolation time is a minimum of 30 days. PCR available. See Molecular Diagnostics section.
Infectious tracheitis	Turkeys	*Mycoplasma meleagridis*	Air sac or exudate	Isolation time is a minimum of 30 days.
Johne's Disease		*Mycobacterium paratuberculosis*	Feces, affected intestine, regional lymph node	At least 5 grams of feces required for culture. Specimens should be shipped within 24 hours of collection. PCR is. See Molecular Diagnostics section.
Leptospirosis		*Leptospira*	Urine	PCR available. See Molecular Diagnostics section
Listeriosis	Bovine	*Listeria monocytogenes*	Cerebellum, pons, medulla, uterine secretions, fetus	Culture may take up to 2 weeks. PCR is available. See Molecular Diagnostics section.
Lumpy jaw	Bovine	*Actinomyces bovis*	Exudate or lesion "sulfur granules"	Collect exudate from non-draining lesion. Ship in anaerobic container.
Lymphadenitis, caseous	Ovine/Caprine	*Corynebacterium pseudotuberculosis*	Affected lymph node	Collect exudate from non-draining lesion
Lymphadenitis, cervical	Porcine	*Streptococcus*	Affected lymph node	Collect exudate from non-draining lesion
Lymphangitis, ulcerative	Equine	*Rhodococcus equi*	Affected lymph node	Isolation time is 2-3 days
Malignant edema	Bovine	*Clostridium spp.*	Lesion	Ship specimen in anaerobic container. FA exam performed. If positive, culture is not done.
Mastitis/routine survey	Bovine	*Various*	See p. 17 for description of collection and care of milk specimens	Specify which agents are suspected—bacteria, yeasts, fungi, *Mycoplasma.* Appointments must be made for surveys.
Mucormycosis		*Mucor sp.*	Lesion	Isolation time is 3-5 days.
Mycotic stomatitis		*Candida albicans*	Affected tissue	Isolation time is variable.
Necrotic enteritis		*Clostridium spp.*	Affected intestine	PCR is available to detect the presence of toxin genes in *Cl. perfringens.*
Nocardiosis		*Nocardia sp.*	Lesion	Culture may take up to 4 weeks.
Omphalophlebitis (Navel ill)		Various opportunis-tic bacteria	Lesion	Isolation time is variable.
Potomac Horse Fever		*Ehrlichia risticii*		Culture not available. See Molecular Diagnostics or Serology sections.
Pneumonia		Various	Lung	Please indicate if *Mycoplasma*, *Hemophilus*, or *Rhodococcus* is suspected.
Proliferative enteritis		*Lawsonia intracellularis*	Intestine/mucosa scraping	PCR only. See Molecular Diagnostics section.
Quail disease		*Clostridium colinum*	Small intestine, liver	Ship specimen in anaerobic container. Difficult to isolate.

Disease	Species	Agent	Preferred Specimen	Comments
Shipping fever complex	Bovine	*Pasteurella multocida, Mannheimia hemolytica,* Others	Lung	Indicate if *Hemophilus* is suspected
Sporotrichosis		*Sporothrix schenckii*	Exudate from lymphatic system	Cultural examination may take up to 4 weeks.
Strangles	Equine	*Streptococcus equi*	Exudate	Collect exudate from non-draining lesion
Swine dysentery	Porcine	*Brachyspira hyodysenteriae*	Colon	6-8 inches of spiral colon from an acutely affected pig. Specimen should be submitted fresh. PCR available. See Molecular Diagnostics Section
Thromboembolic meningoencephalitis (TEME)	Bovine	*Hemophilus somnus*	Affected organ	Isolation time is 3-7 days.
Trichomoniasis		*Trichomonas fetus*	Vaginal mucous	ADDL does direct examination only
Tuberculosis		*Mycobacterium tuberculosis*		Sent to NVSL. Call ADDL for instructions on preparing tissue for shipment to NVSL.
Tyzzer's Disease		*Clostridium piliformis*		Diagnosed by histopathology Culture not available.
Ureaplasma		*Ureaplasma*	Urine, aborted fetus, affected tissue	Submit as soon as possible.

Molecular Diagnostics

Definition: A test designated as PCR is a polymerase-chain-reaction test to detect DNA and is composed of three basic parts:

1) Extraction of the DNA from the sample,
2) Addition of sample DNA, one set of DNA nucleotide primers and other reagents to a PCR Cycler machine for amplification of target DNA, and
3) Detection of target DNA by gel-electrophoresis

A test designated as RT-PCR is a reverse-transcriptase PCR test to detect RNA and is composed of the same three basic parts as PCR with the additional step using reverse-transcriptase enzyme to synthesize complementary DNA from the target RNA. The complementary DNA is then run in the PCR test.

Nested PCR is a modification that uses two sets of nucleotide primers and two complete cycles of amplification; the second cycle of amplification further amplifies a target fragment of RNA originating within an already amplified larger target fragment of DNA. Nested PCR results in higher sensitivity than simple PCR or RT-PCR and is used for diseases that have very little target nucleic acid in tissue samples.

In Real-time PCR, amplification and detection of target sequences are achieved simultaneously in a single tube. Real-time PCR reaction mixtures contain a single-strand DNA probe that can specifically hybridize with the target sequences that are being amplified. The probe is labeled with a fluorescent dye at one end and a quencher molecule at the other end. Since the probe hybridized to its specific target sequences gives stronger fluorescent signal than the unhybridized probe, the intensity of fluorescent signal detected during PCR is directly proportional to the amplification of the specific DNA fragment. The advantages of real-time PCR include higher assay specificity, quicker turnaround time, and the potential for quantitative results.

PCR Requests

PCR tests are run on request only. Additional charges will be incurred for each PCR test that is run on samples mailed to ADDL and on tissues collected during necropsy examination at ADDL. When PCR tests are desired for various agents, each desired agent must be clearly requested on the ADDL submission form. Alternatively, and only for animals submitted for necropsy, permission may be granted by checking the box on the submission for “For necropsy cases, permission is granted to run PCR tests deemed necessary by an ADDL pathologist.”

- Prior arrangements are required for submission of >10 samples and for swine semen samples.

- It is best to collect samples with disposable instruments (plastic tableware often works well) into Sterile Whirl Pak bags. Optimum sample size per test is approximately 10-15 grams or mls. Eliminate air, roll the tops down, and seal by folding tabs. Avoid use of instruments that may be contaminated from previous use.

- Tissues sent for PCR must be packaged separately in Whirl Paks and clearly labeled as to tissues included and tests requested. Samples should be shipped overnight on ice packs in an insulated shipping container. All tissue samples received in a single bag will be pooled, and a single PCR test will be run for each requested agent. If the same PCR test is desired on tissues from different animals, or from different tissues from the same animal, samples must be submitted in separate Whirl Pak Bags.

Agent	Test Type	Preferred Specimen
Avian influenza	Real-time PCR	Tracheal swab, cloacal swab
BVD	Real-time PCR	lymph nodes, spleen, serum, blood, ear notches, pooled ear notches (See Virology Section, p. 45)
BVD1, BVD2 typing	RT-PCR, nested	Lymph nodes, spleen, serum
Brachyspira hyodysenteriae	Real-time PCR	Cecal and colonic mucosa, feces
Brachyspira pilosicoli	Real-time PCR	Cecal and colonic mucosa, feces
Chlamydia sp. (genus specific)	PCR	Placenta, liver
Chlamydia psittaci	PCR	Placenta, liver, other
Circovirus 2, Porcine	Real-time PCR	Serum/plasma, lymph nodes, tonsil, spleen, kidney, liver
Classical Swine fever virus	Real-time PCR	tonsil
Clostridium difficile toxins A,B	PCR	Frozen colonic contents from animals with minimal autolysis
Clostridium perfringens typing panel (a,b,e,I,b2 toxins, enterotoxins)	PCR (multiple)	Bacterial isolates only; first requires bacterial culture
Coronavirus, Bovine	RT-PCR	Feces, intestinal contents, nasal swab
Eastern equine encephalitis virus	Real-time PCR	brain, spinal cord, spinal fluid
Equine herpesvirus I	Real-time PCR	Nasal swab, tracheal wash, CSF, placenta, lung, liver
E. coli K88 or K99	PCR	Bacterial isolates only, first requires bacterial culture
E. coli virulence typing panel: F18, 987:, F41 pili, EAE attachment gene, LT, Sta;, Stb	PCR	Bacterial isolates only, first requires bacterial culture
Enteric panel (swine) Includes *Salmonella*, Lawsonia *intracellularis, B. pilosicoli, B. hyodysenteriae*	Real-time PCR	Small intestine, feces
Influenza A virus	Real-time PCR	Nasal swabs, tracheal swabs, cloacal swabs, lung
Influenza viral typing; H1N1, H1N2, H3N2	RT-PCR, 2 tests	Viral isolates only; first requires virus isolation
Lawsonia intracellularis	Real-time PCR	Feces, small intestine (ileum)
Leptospira sp. (all pathogenic species)	Real-time PCR	Urine, liver, kidney
Listeria monocytogenes	PCR	Bacterial isolates only
Mycobacterium paratuberculosis	PCR	Ileal mucosa, colon, ileocolic valve, mesenteric lymph nodes, feces
Mycoplasma gallisepticum	PCR	Lung, trachea, oviduct, choanal/tracheal/sinus swabs
Mycoplasma hyopneumoniae	Real-time PCR	Nasal/bronchial swabs, bronchial alveolar lavage fluid, lung tissue
Mycoplasma synoviae	PCR	Lung, trachea, oviduct, choanal/tracheal/sinus/joint swabs
Myxobolus cerebralis	PCR nested	Hemi-section of head
Neospora caninum	Real-time PCR	Any tissue with lesions
Parvovirus—canine/feline	Real-time PCR	feces, any tissue with lesions
PRRS Virus (detects both US and European strains)	Real-time PCR	Serum, spleen, lung, semen, lymph nodes, tonsil
Potomac Horse Fever	Real-time PCR	Lymph node, spleen, intestinal contents
Q fever (*Coxiella burnetii*)	Real-time PCR	Vaginal swabs, placenta, fetal lung, fetal intestinal and stomach contents
Renibacterium salmonarium	PCR	Kidney
Salmonella (genus specific)	Real-time PCR	Intestinal mucosa, feces, other tissues as indicated
TGE (Transmissible gastroenteritis)	Real-time PCR	Small intestine, feces
TGE Virus (differentiates from Porcine Respiratory Corona Virus)	RT - PCR, nested	Small intestine, feces
Toxoplasma gondii	Real-time PCR	brain, liver, spleen, lung
West Nile virus (mammals)	RT- PCR, nested	Brain, spinal cord
West Nile Virus (avian)	RT -PCR	Kidney, heart, brain, liver, spleen

Pathology

Procedure	Specimen	Container	Comments
Necropsy	Maximum number of animals (similar age and clinical problem) to be included in 1 accession: Cattle: 1 adult or 2 calves Cats: 1 adult or 3 kittens Dogs 1 adult or 3 puppies Horses: 1 adult or 2 foals Sheep: 1 adult or 3 lambs Swine: 1 adult or 3 pigs Abortion/Stillbirth: 3 fetuses w/placenta	Shipping containers for mail or courier services must be leak-proof and airtight	-All species -Carcasses must be held at refrigeration temperature until delivery to ADDL. -DO NOT freeze unless interval between death and delivery > 3 days. -Carcasses smaller than 150 lb. can be delivered after hours to the walk-in refrigerator on the south dock (be sure to identify with name, contact phone number). -Gross necropsy results will be reported in 1-5 working days; final reports are completed within 5-14 working days. *-ADDL does not euthanize companion animals, including horses.*
Cosmetic necropsy	1 animal		-Prior arrangements with ADDL are required. However, cosmetic necropsy is discouraged because it prevents thorough postmortem examination and may hinder diagnosis. -Animal must be in ADDL before 1 pm and will usually be returned to owner the same day. Additional charge applies.
Legal/Insurance Necropsy	1 animal		Must be designated as such prior to necropsy. Additional charge applies.
Field Consultation			Call to schedule. Field consultations are made only at the request of a veterinarian.
Practitioner-performed necropsy	-Brain: whole or half -Spinal cord: entire, with opened dura -Eyes: whole -Endocrine glands: small, whole; larger glands, bisected -Heart, lung, liver, kidney, spleen: 5 mm thick slices (whole heart if cardiac defect is suspected) -GI tract: 3-5 cm segments	10% formalin	-Brain, heart, lung, liver, kidney and spleen plus any other tissues with gross lesions should be submitted from all necropsy examinations. Other tissues should be included according to history or clinical signs. -Formalin-fixed specimens accompanied by fresh (unfixed) specimens for ancillary testing (microbiology, toxicology, virology etc) are processed as long-form accessions. Histologic findings are reported in 3-5 working days with final reports in 7-15 days.
Amputations	-Small specimens, e.g. digits, may be submitted whole -Larger specimens, e.g. limbs, should be refrigerated and delivered to ADDL within 24 hours	10% formalin -Refrigeration for large specimens	Bony specimens will require 1-10 day delay for decalcification. Additional charges apply. -Limb amputations can be submitted as a "Biopsy Plus" for dissection and gross examination by a pathologist before histopathology. These will be charged as a necropsy.
Histopathology	Various tissue specimens	Leak-proof wide-mouth jar or Whirl Pak	-Tissues should be fixed in 10% buffered formalin with 10:1 formalin to tissue ratio. -Excess formalin can be decanted after 1-2 days fixation.
Endoscopic, pinch, core (Tru-cut), or punch biopsies	Entire biopsy specimen: if <3mm in any dimension, submit in histologic cassette to avoid specimen damage or loss	10% formalin	Multiple specimens should be submitted in individually labeled containers to indicate tissue site. Histologic findings from Neoplasm Submission Form accessions are reported in 1-3 working days. Histochemistry or immunohistochemistry requires another 1-5 working days.
Excisional biopsies	If >2cm in diameter, slice accordingly to allow fixation	10% formalin	Use paint or suture tags to orient specimen or identify margins, if pertinent. Histologic findings from Neoplasm Submission Form accessions are reported in 1-3 working days.

Histopathology

- Tissue sample should be no more than 0.5 cm thick and placed in a wide mouth jar containing 10% buffered formalin fixative.
- For fixation, the formalin amount should be at least 10X the volume of the sample.
- Alternatively, tissues may be fixed for 24-48 hours and then transferred to a Whirl Pak bag with enough formalin to prevent drying.
- Tissues should arrive at ADDL already fixed in formalin.
- If formalin is not used as the fixative, please indicate which fixative was used.
- Before shipping, contact the postal service or courier service to be sure to comply with their regulations.
- Histopathology mailers are available from ADDL. See Page 5 of this manual.

Painting the Surgical Margins of a Tumor Biopsy

A common problem faced by pathologists is interpreting surgical margins of tumor biopsies. Although it is often easy to distinguish surgical margins from those produced during the trimming process in the histology laboratory, it is sometimes difficult and frustrating for both pathologists and clinicians. Diagnosticians know that the three most common questions posed by the clinician regarding a neoplastic process are “What kind of tumor is it?,” “Is it benign or malignant,” and “Are the margins free of neoplastic cells?”

In human medicine, surgical margins of a biopsy are commonly painted with a dye that adheres to tissue and is visible under the microscope. The procedure is simple and does not interfere with histologic evaluation. Its advantage is that it clearly distinguishes surgical margins from trimming margins. This is essential to assess complete/incomplete excision of a tumor.

Biopsy margin painting can be done on unfixed or fixed tissues; however, painting unfixed tissues is easier and preferred. There are several commercially available products for this purpose (call the ADDL for additional information, if needed). The use of different colors for different aspects of mass orientation is superior to using sutures of different colors. Painting biopsy margins is also inexpensive; a 20 ml bottle of dye will last several years.

Check the following URL for additional information: <www.addl.purdue.edu> and click on Winter 2004 newsletter.

How to Paint the Surgical Margins of a Tumor Biopsy

1. Blot the biopsy margins of the mass.
2. Select the dye color.
3. With a wooden applicator stick or cotton swab, “paint” the biopsy margin.

   Do not pour dye on the surface; apply as if painting.
4. Let the dye dry for 5-10 minutes, and immerse the sample in regular fixative solution. Some of the dye will dissolve with the fixative; this will not affect the sample. If the sample is thicker than 4-6 mm, section it to improve penetration of fixative as you usually do.

Immunohistochemistry

Immunohistochemistry (IHC) uses immunologic and histologic techniques to detect antigens in tissues. The antigen is recognized by a specific antibody that is added to the tissue section. The immunologic reaction is visualized under the microscope by adding an enzyme, a substrate to the enzyme, and a chromogen, producing a colored reaction. IHC is a very sensitive and specific technique. For diagnosticians, it is an important technique that allows us to colocalize antigens and lesions in the same tissue section.

Neoplastic and infectious diseases are the main focus of IHC in veterinary medicine. The ADDL IHC Service offers a variety of tests for both infectious and neoplastic diseases.

Diagnosis of neoplasia: Often, the tissue of origin of a tumor cannot be determined with routine histology. Using specific antibodies for different tissues or cells (e.g. cytokeratin for epithelium, vimentin for mesenchymal cells, lymphoid markers, etc), the origin of many tumors can be determined with IHC.

Diagnosis of micrometastases: Early metastasis can be difficult to detect using conventional histology. IHC highlights the presence of single or small groups of neoplastic cells in metastatic sites. Early detection of micrometastases increases the chances of survival with surgical removal of affected nodes or by modification of the treatment protocol.

Prognostic markers: Some proteins are expressed in neoplastic, but not in normal, mature cells (e.g., embryonal proteins), expressed in neoplastic cells in larger amounts than in normal cells (e.g. cycle-related proteins), or structurally modified in neoplastic cells (mutant p53 protein). These changes may have prognostic significance in specific tumor types. For instance, it has been reported that the immunohistochemical detection of c-Kit protein in mast cell tumors of dogs has prognostic significance. Some of these markers are being tested to determine their significance in veterinary cancers.

Diagnosis of infectious diseases: Detection of antigens of an infectious agent using IHC has etiologic significance. Antigen detection can be correlated with histopathologic changes and thus can confirm the significance of a particular microorganism detected by other methods.

How to Submit Samples for Immunohistochemical Testing

- **We test samples that have been fixed in formalin, so you do not have to do anything special.**
- **Submit the sample as you would for routine histopathology.**
- **Do not hold fixed samples in your office longer than 2 days as prolonged fixation may destroy antigens. As soon as you place your sample in formalin, send it to the ADDL.**

Tissue/ Cell Marker	Use
Actin, muscle	All muscle
Actin, sarcomeric	Striated muscle
Actin, smooth muscle	Smooth muscle
B-cell marker (BLA36)	B-cell lymphoma, some histiocytic tumors
CD3	T-cell lymphoma
CD10	Renal tubular epithelium
CD11d	Some histiocytic tumors
CD18	Leukocytic tumors
CD20	B-cell lymphoma
CD31	Vascular tumors
CD45	Leukocytic tumors
CD68	Histiocytes
CD79a	B-cell lymphoma
CD117 (c-Kit protein)	Mast cell tumors
Calcitonin	C-cell tumors of thyroid gland
Calretinin	Renal tubules, nerve tissue
E-Cadherin	Langerhan cells, epithelium, histiocytomas
Caspase-3	Apoptotic cells
Chromogranins A+B	General neuroendocrine marker
COX-2	Carcinomas
COX-1	Normal urothelium, endothelium
Cytokeratin 5	Myoepithelium, basal cells
Cytokeratin 7	Glandular epithelium
Cytokeratins (MNF and AE1/AE3)	General epithelial marker
Cytokeratins high molecular weight	Squamous epithelium, mesothelium, hepatocytes
Desmin	Muscle tumors
Estrogen receptor	Estrogen receptor-positive tissues and tumors
Factor VIII-related antigen	Vascular tumors
Glial fibrillary acidic protein	Neural (glial) tumors
Glucagon	Pancreatic islet tumors
Glucose transporter (Glut-1)	Peripheral nerves, kidney
Hepatocyte marker 1	Hepatocytes and their tumors
Immunoglobulin kappa chains	Plasmacytomas, immunocomplex disease
Immunoglobulin lambda chains	Plasmacytomas, immunocomplex disease

Tissue/ Cell Marker	Use
Inhibin-alpha	Sex-cord testicular and adrenal cortical tumors
Insulin	Insulin-producing tumors
Ki-67	Cell proliferation
Lysozyme	Histiocytes
Melan A	Melanomas, steroid-producing tumors
MHC II	Antigen presenting cells, lymphocytes
Microphthalmia transcription factor	Melanomas
MUM1	Plasmacytomas, some B-cell tumors
Myoglobin	Striated muscle
Myeloid-histiocytic marker	Macrophages, myeloid cells
Natural killer	Peripheral nerve cell tumors
Nerve growth factor receptor	Neural tumors
Neurofilament	Neural tumors
Neuron specific enolase	General neuroendocrine marker
p53	p53 abnormal (mutated) detection only
p63	Myoepithelium, basal cells
PGP 9.5	General neuroendocrine marker
Progesterone receptor	Positive tissues/tumors
S-100 protein	General neural marker, neuroendocrine tumors
Somastotatin	Pancreatic islet cell tumors
Synaptophysin	General neuroendocrine marker
Thyroglobulin	Thyroglobulin-producing cells
Thyroid transcription factor 1	Lung/thyroid tumors
Tryptase	Mast cell tumors
Uroplakin III	Urothelium and its tumors
Vimentin	General marker for mesenchymal tumors

Infectious Diseases
Adenovirus
Aspergillus
Bovine respiratory syncytial virus
Bovine virus diarrhea
Calicivirus
Coronavirus, bovine
Coronavirus, porcine
Coronavirus, feline
Cryptosporidium parvum
Distemper virus
Feline leukemia virus
Francisella tularensis
Herpesvirus 1, bovine
Herpesvirus 1, equine
Herpesvirus 1, feline
Influenza A
Lawsonia
Leptospira
Listeria
Mycobacterium bovis
Neospora caninum
Papilloma virus
Parvovirus, canine
Porcine circovirus 2
PRRS virus
Rabies
Rotavirus A
Sarcocystis neurona
Toxoplasma gondii
West Nile Virus
Yersinia pestis

IHC for Major Tumor Groups

Tumor Type	Markers
Adrenal	Melan A and inhibin-alpha (cortex); PGP 9.5 and synaptophysin (medulla)
Endocrine (generic)	PGP 9.5, chromoganin A, synaptophysin, neuron specific enolase, S100
Epithelial vs. mesenchymal	Cytokeratins (epithelial), vimentin (mesenchymal), E-cadherin (epithelial), p63 (basal cells, myoepithelium)
Lymphoid	CD3 (T-cell), CD79a and CD20 (B-cell), CD45 and CD18 (panleukocytic), MUM1 (plasma cells)
Leukocytic	CD45 (panleukocytic), CD18 (with emphasis in histiocytic), CD11d (dendritic cells), E-cadherin (Langerhan's cells, histiocytoma), myeloid histiocytic marker (macrophages, myeloid cells)
Liver	HepPar1 (hepatocytes), cytokeratin 7 (bile duct epithelium)
Mast cell tumors	CD117, tryptase
Melanocytic tumors	Melan A, S100, NSE
Muscle differentiation	Actin muscle (all), actin smooth muscle, myoglobin (striated muscle), actin sarcomeric (striated muscle), desmin (muscle)
Neurogenic tumors	S100, neurofilament, natural killer, glut1, nerve growth factor receptor
Pancreas (endocrine)	Insulin, glucagon, somastotatin
Squamous vs. adenocarcinoma	Squamous cell carcinoma (CK5, p63) Adenocarcinoma (CK7)
Testicle	Sex-cord tumors (inhibin-alpha), germ cell tumors (KIT)
Thyroid	TTF1 (follicles and medulla), calcitonin (medulla, C-cells); thyroglobulin (follicles)
Urinary tumors	Uroplakin III, cytokeratin 7, COX-2, COX-1
Vascular tumors (endothelium)	Factor VIII-related antigen, CD31

Serology

General Guidelines and Information

1. All forms must be complete and legible.

2. All regulatory charts must include the submitting veterinarian's signature.

3. All regulatory charts must include complete animal identification.

4. "Buckslips" must accompany all regulatory charts (available from ADDL).

5. Advance notice should be given when submitting large groups of serum samples.

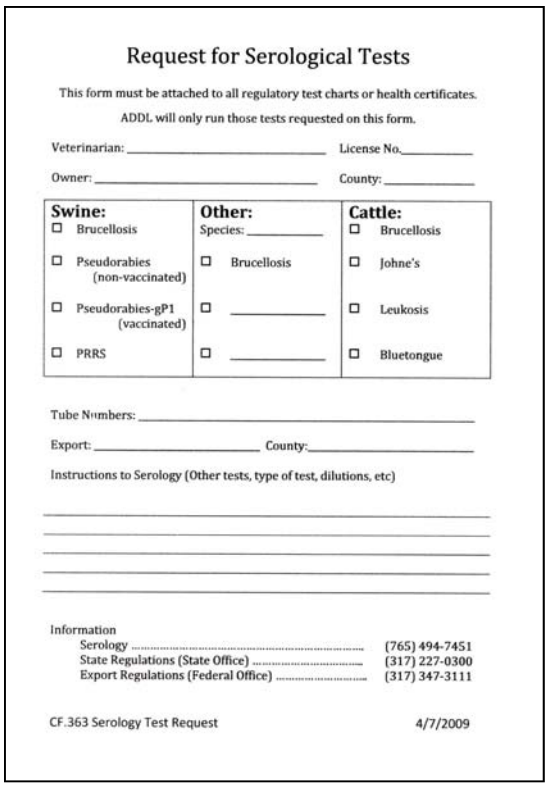
6. Samples delivered to the ADDL Serology Laboratory by the owner must be sealed using the veterinarian's label or tape bearing the veterinarian's signature.

7. Be aware that verification of any non-negative samples will cause a delay in the availability of results.

8. Samples must be received before noon on the day BEFORE the test is scheduled to be set (started). Please see the pages 31–33for individual test schedules.

9. If multiple tests are requested, allow extra time for completion.

"Buckslip"



Request for Serological Tests

This form must be attached to all regulatory test charts or health certificates.
ADDL will only run those tests requested on this form.

Veterinarian: _____ License No._____

Owner: _____ County: _____

Swine:	Other:	Cattle:
☐ Brucellosis	Species: _____	☐ Brucellosis
☐ Pseudorabies (non-vaccinated)	☐ Brucellosis	☐ Johne's
☐ Pseudorabies-gP1 (vaccinated)	☐ _____	☐ Leukosis
☐ PRRS	☐ _____	☐ Bluetongue

Tube Numbers: _____

Export: _____ County:_____

Instructions to Serology (Other tests, type of test, dilutions, etc)

Information
Serology ..... (765) 494-7451
State Regulations (State Office) ..... (317) 227-0300
Export Regulations (Federal Office) ..... (317) 347-3111

CF.363 Serology Test Request 4/7/2009

10. Testing schedules may change to accommodate caseloads and/or holidays.

11. Tests performed at the National Veterinary Services Laboratory (Ames, Iowa) must be submitted through ADDL.

12. Exports—the submitting veterinarian is responsible for informing ADDL of any special requirements (e.g., dilutions, test method), using ADDL Form 3 (buckslip). If this information is not supplied, the standard test will be performed.

13. Test regulations—for information please call:

Federal-USDA Area Veterinarian in Charge	Office of the State Veterinarian
(317) 347-3100	(317) 227-0300

14. If there are any questions concerning submissions, please contact the ADDL Serology office at (765) 494-7451 prior to submitting samples.

Submitting Serum Samples to ADDL

1. Submitting clear serum in 5 ml cap tube (size 12x75) is preferred.
2. Each tube must be identified by at least a tube number and contain at least 1 ml of clear serum; further identification is preferred.
3. Tube numbers and numbers on chart must match and be in consecutive order (no skipped numbers).
4. Acute and convalescent samples of serum should be submitted together. ADDL will not hold single samples pending a later submission.

Acceptable Forms for Submitting Serum Samples to ADDL

1. Form VS4-33 Brucellosis Test Record
   Usage: All regulatory testing
   Supplied by: State Board of Animal Health (317-227-0300)

2. Certificate of Veterinary Inspection (Health Certificate)
   Usage: All show, interstate movement and sale testing
   Supplied by: State Board of Animal Health (317-227-0300)

3. Form VS10-11 Equine Infectious Anemia Laboratory Test Record
   Usage: EIA testing only
   Supplied by: State Board of Animal Health (317-227-0300)

4. ADDL Form 1, Accession Form
   Usage: All diagnostic testing
   Pages 1 and 3 of this form must be completed
   Supplied by: Animal Disease Diagnostic Laboratory
   Downloadable version at www.addl.purdue.edu

5. ADDL Form 3, Request for Serologic Tests "Buckslip"
   Usage: Must be completed and attached to all regulatory test records and health certificates (ADDL will run ONLY those tests requested on this form. It is not necessary to attach a buckslip to ADDL Form 1 [diagnostic testing], or EIA test charts
   Supplied by: Animal Disease Diagnostic laboratory

Test Abbreviations

AGG	Agglutination
AGID	Agar gel immunodiffusion
BAPA	Buffered acidified plate antigen
CF	Complement fixation
ELISA	Enzyme Linked Immunosorbent Assay
cELISA	Competitive Enzyme-Linked Immunosorbent Assay
HI	Hemagglutination inhibition
IFA	Indirect fluorescent antibody assay
LAT	Latex agglutination
MA	Microagglutination
RIV	Rivanol
SA	Slide agglutination
SPT	Standard plate test
STT	Standard tube test
VN	Virus neutralization
IPT	Immunoperoxidase test

Agent	Test method	Day set	Comments
Abortion screen—bovine			Brucellosis Infectious Bovine Rhinotracheitis Virus (IBR) Bovine Viral Diarrhea virus (BVD) Parainfluenza virus 3 (PI3) Leptospirosis (Lepto) Neospora
Abortion screen—equine			Equine herpesvirus 1 (EH1) Equine arteritis virus (EAV) Leptospirosis (Lepto)
Abortion screen—ovine/caprine			Bovine Viral Diarrhea virus (BVD)/ Border Disease Virus (BDV) Bluetongue Virus (BTV) Toxoplasma Leptospirosis (Lepto)
Abortion screen—porcine			Brucellosis Pseudorabies (PrV) Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Porcine Circovirus (PCV) Parvovirus (PPV) Leptospirosis (Lepto)
Anaplasmosis	cELISA	As needed	Positive/negative result
Bluetongue	cELISA	As needed	Positive/negative result
Brucella abortus	BAPA, Card, SPT (plate), RIV, STT (tube)	M-F	STT tube test set M, T, W only
Brucella canis	SA	M-F	Positive/negative result
Bovine Leukosis Virus	cELISA	As needed	Positive/negative result
	AGID	M-F	Positive/negative result
Bovine Respiratory Syncytial Virus	VN	W*	Titer result
Bovine Viral Diarrhea Virus Type 1	VN	M, Th*	Titer result
	ELISA	As needed	Positive/negative result
Bovine Viral Diarrhea Virus Type 2	VN	M, Th*	Titer result
	ELISA	As needed	Positive/negative result
Caprine Arthritis Encephalitis	AGID	M-F	Positive/negative result
	cELISA	As needed	Positive/negative result
Canine Distemper Virus	IFA	As needed	Positive/negative result; titration on request
Canine Herpesvirus	IFA	As needed	Positive/negative result; titration on request
Canine Parvovirus	IFA	As needed	Positive/negative result; titration on request
Equine Arteritis Virus	VN	T*	Titer result
Equine Herpesvirus 1-4 (Rhinopneumonitis)	VN	W*	Titer result
Epizootic Hemorrhagic Disease	AGID	M-F	Positive/negative result
Equine Infectious Anemia	cELISA	M-F	Positive/negative result
	AGID	M-F	Positive/negative result

* *Sample must be received at ADDL by noon on the day prior to test day*

Agent	Test method	Day set	Comments
Feline coronavirus (FIP)	IFA	As needed	Positive/negative result; titration on request
Feline Parvovirus (Panleukopenia)	IFA	As needed	Positive/negative result; titration on request
Infectious Bovine Rhinotracheitis (IBR)	VN	M,Th*	Titer result
	cELISA	As needed	Positive/negative result
Johne's (M. *paratuberculosis)*	ELISA	As needed	Positive/negative result
Leptospirosis (7 serovars)	MA	M	Serovars: autumnalis, bratislava, canicola, grippo, hardjo, ictero, pomona Titer result
Mycoplasma hyopneumoniae	ELISA	As needed	Positive/negative result
Neospora caninum	ELISA	As needed	Positive/negative result detects antibodies in bovine serum
Ovine Progressive Pneumonia	AGID	M-F	Positive/negative result
Parainfluenza 3 Virus (PI3)	VN	W*	Titer result
Porcine Circovirus	IFA	As needed	Positive/negative result; titration on request
	ELISA	As needed	Positive/negative result
Porcine Cytomegalovirus	IFA	As needed	Positive/negative result; titration on request
Porcine Enterovirus types 1-7	VN	T,F*	Titer result
Potomac Horse Fever	IFA	As needed	Positive/negative result; titration on request
Porcine Parvovirus	IFA	As needed	Positive/negative result; titration on request
	cELISA	As needed	Positive/negative result
Porcine Respiratory Coronavirus (PRCV) and Transmissible Gastroenteritis (TGE) differential	ELISA	As needed	Positive/negative result
Pseudorabies Virus (PRV)	gBcELISA	As needed	Positive/negative results
	VN	T,F*	Titer
Pseudorabies Virus (gP1 deleted gene)	cELISA	As needed	Positive/negative result
Porcine Reproductive and Respiratory Syndrome Virus (PRRSV- European/ North American strains)	ELISA	As needed	Positive/negative result
	IFA	As needed	Positive/negative result; titration on request
Rotavirus	IFA	As needed	Positive/negative result; titration on request
Swine Influenza Virus (H1N1, H3N2)	HI	As needed	Titer result
	ELISA	As needed	Positive/negative result
Transmissible Gastroenteritis Virus (TGE)	VN	T,F	Titer result
Toxoplasma	IFA	As needed	Positive/negative result; titration on request
Vesicular Stomatitis	VN	T,F*	Indiana, New Jersey strains; Titer result

** Serum must be in the lab by noon on the day prior to test day*

Serum submitted to Purdue ADDL for the following tests will be forwarded to the National Veterinary Services Laboratory in Ames, Iowa.

Test	Procedure
Brucella melitensis	3% card
Brucella ovis	ELISA CF
Malignant Catarrhal Fever	VN IPT
Q fever	CF
Equine Influenza (Prague, KY, Miami)	HI
Equine Encephalomyelitis (EEE,WEE,VEE)	VN EEE also by IgM ELISA
West Nile Virus (WNV)	IgM ELISA VN

Toxicology/Analytical Chemistry

General Guidelines for Toxicology/Analytical Chemistry Submissions

1. Refrigerate or freeze tissue samples.
2. Include a complete history.
3. Test requests must be specific. If you are unsure of what test to request, call the Toxicology Laboratory prior to sample submission.
4. Samples will be held for 2 weeks past the final report date of Toxicology testing. Please notify the Toxicology Laboratory as soon as possible to request additional testing.

Suspected Toxicoses of Unknown Origin

When a toxicosis is suspected, but the specific toxin is unknown, please follow these guidelines:

1. Obtain a complete history.
2. Perform a complete physical examination or necropsy (depending on the state of the animal).
3. If the animal is alive, collect, freeze, and save:
    - A) Vomitus (all available)
    - B) Urine (all available)
    - C) Whole blood (5 ml in red top clot tube)
    - D) Whole blood (5 ml in purple top EDTA tube)
    - E) Serum in glass tube with NO rubber stopper (if zinc toxicity is suspected)
4. If at necropsy:
    - A) Fix representative tissues in 10% formalin
    - B) Save samples for bacteriology and virology if appropriate
    - C) Freeze and save the following for possible toxicology testing
        - i. Liver (approximately 100 g)
        - ii. Stomach/rumen contents (approximately 100 g or all available)
        - iii. Brain (if organophosphate toxicosis is suspected) 1/2 brain from large animals; whole brain from small animals
        - iv. Kidney (1/2)
        - v. Urine (all available)
        - vi. Eyeball or ocular fluid (if nitrate toxicosis is suspected)
5. If feed and/or water are possibly related, submit:
    - A) A minimum of 1 pound of feed
    - B) A minimum of 1 quart of water
6. Call the ADDL for consultation on appropriate testing based on the clinical history and physical examination or necropsy findings.

Test	Preferred Specimen	Container	Time	Comments
Acetylcholinesterase activity (Organophosphate/ Carbamate indicator)	-Frozen brain (1/2 brain or entire brain from small animal) -Blood in EDTA	Whirl Pak Glass tube	1 d 1 d	Freeze brain samples. Include list of insecticides available on premises.
Aflatoxin	Feed	Paper bag		See Mycotoxin Screen 1.
Alkaloid screen	-Bait (20 g) -Ingesta (20 g) -Liver (20 g) -Urine (40 ml)	Whirl-pak Whirl-pak Whirl-pak Glass jar	Call lab	Freeze or refrigerate sample. Call ADDL for test availability.
Anticoagulants	-Forage (5 lbs) -Blood (5 ml) -Liver (10 g)	Paper bag EDTA tube Whirl Pak	3 w 3 d 3 d	 Freeze or refrigerate sample. Freeze sample.
Bone screen	Femur		1 w	Free of soft tissue. Screen includes ash, calcium, density, phosphorus.
Bone marrow fat content	Femur	Plastic bag	2 d	For small animals, send both left and right femurs
Carbamates				See Organophosphate screen.
Chlorinated hydrocarbons	-Feed (1 lb) -Ingesta (100 g) -Water (1qt) -Brain -Whole blood (5 ml)	Paper bag Whirl pak Glass jar Whirl Pak EDTA tube		Include list of available insecticides. Include list of available insecticides. Include list of available insecticides. Freeze or refrigerate. Include list of available insecticides.
Copper	-Liver (20 g) -Clear serum (2 ml) -Feed (1 lb) -Kidney (20 g)	Whirl Pak Glass tube Paper bag Whirl Pak	2 d 1 d 2 d 2 d	Freeze or refrigerate. Freeze or refrigerate. Freeze or refrigerate.
DAS (Diacetoxyscirpenol)				See Mycotoxin Screen 2.
Drugs				Call Toxicology lab prior to submission.
Ethylene glycol	-Serum (5 ml) -Kidney (10 g) -Kidney (slice in formalin)	Glass tube Whirl Pak		Qualitative tests at ADDL. Sent to Michigan State Microscopic examination
Fumonisin	Feed (1 lb)	Paper bag	2-3 d	
Ionophore screen—Includes monensin, lasalocid, salinomycin	-Feed (1 lb) -Ingesta	Paper bag Whirl Pak	2-3 d 2-3 d	 Freeze or refrigerate samples. Qualitative test at Purdue ADDL Quantitative test sent to UC Davis
Iron	-Liver (20 g) -Whole blood (5 ml) -Clear serum (5 ml)	Whirl Pak EDTA tube	3 d	Freeze or refrigerate
Lasalocid				See Ionophore screen
Lead	-Kidney (1/2 or all of small kidney) -Liver (20 g) -Paint chips (5 g) -Whole blood (2-5 ml)	Whirl Pak Whirl Pak Whirl Pak EDTA tube	2 d 2 d 2 d 1 d	Freeze or refrigerate Freeze or refrigerate Heparinized or EDTA blood required
Methylxanthines (Caffeine, Theobromine, Theophylline)	Serum/plasma (5ml)	Glass test tube	2 d	Submit refrigerated or frozen
Monensin				See Ionophore screen
Mycotoxin Screen 1	Feed (1 lb)	Paper bag	2 d	Ochratoxin, DON, aflatoxin, zearalenone Qualitative
Mycotoxin Screen 2	Feed (1 lb)	Paper bag	2 d	T-2, DAS Qualitative

Test	Preferred Specimen	Container	Time	Comments
Nitrates	Forage Ocular fluid/serum	Paper bag Glass tube	3-4 d 2-3 w	Refrigerate Sent to Nebraska Diagnostic Lab
Organophosphate/Carbamate screen	-Feed (1 lb) -Ingesta (100 g) -Water (1 qt) -Brain -Whole blood (5 ml)	Paper bag Whirl Pak Glass jar Whirl Pak EDTA tube	3-5 d	Include list of available insecticides. Include list of available insecticides. Include list of available insecticides. See Acetylcholinesterase activity Include list of available insecticides
pH measurement	-Ingesta (20 ml) -Water (10 ml)	Whirl Pak Glass jar	1 d 1 d	Freeze ingesta
Plant and seed identification	-Ingesta -Seed, seed pods -Whole plant	Whirl pak Paper bag Paper bag	3 d 3 d 3 d	Refrigerate ingesta Sent to Purdue Plant and Pest Laboratory Sent to Purdue Plant and Pest Laboratory
Polychlorinated biphenyls (PCBs)	-Fat (10 g) -Milk (250 g) -Silo scrapings	Whirl Pak Glass jar	3 d 3 d 3 d	Place in Whirl Pak Freeze or refrigerate. STORE SAMPLE IN GLASS CONTAINER.
Salinomycin				See Ionophore screen.
Salt (sodium ion)	-Clear serum (3 ml) -CSF (3 ml) -Brain (5 g) -Feed (1 lb) -Water	Glass tube Glass tube Whirl Pak Paper bag Glass jar	1 d 1 d 1 d 1 d 1 d	No hemolysis. Refrigerate CSF. No hemolysis Refrigerate or freeze.
Selenium	-Clear serum (3 ml) -Frozen liver	Glass tube Whirl Pak	1w	Refrigerate. Freeze.
Selenium/Vitamin E	-Clear serum (5 ml) -Frozen liver	Glass tube Whirl Pak	2 w	Refrigerate. Freeze.
Strychnine	-Bait (20 g) -Ingesta (20 g) -Liver (20 g) -Urine (40 ml)	Whirl-pak Whirl-pak Whirl-pak Glass jar	1 w	Freeze sample.
Sulfur	-Feed (100g) -Liver (10gm)* -Water (100ml)	-Whirl-pak -Whirl-Pak -Clean glass bottle or container	3-5 d 3-5 d 3-5 d	* Tissues other than liver, call laboratory prior to submission
T-2 toxin				See Mycotoxin screen 2.
Vitamin E	-Clear serum (5 ml) -Frozen liver (50 g)	Glass tube Whirl Pak	2 d 2 d	No hemolysis. Do not use separator tube. Freeze sample Protect all samples from light
Vomitoxin (DON)				See Mycotoxin Screen 1
Water quality screen	Water (1 qt)	Glass jar	2 d	Bottle must be clean. Refrigerate and transport overnight. Screen includes nitrates, nitrites, chlorides, pH, hardness, alkalinity, carbon dioxide (for fish), ammonia nitrogen. Can include sulfates, sulfides and iron upon request.
Zearalenone				See Mycotoxin screen 1
Zinc	-Clear serum (5 ml) -Liver (20 g)	Glass tube Whirl Pak	1 d 3 d	Sample must not come into contact with rubber stoppers.

Virology

Submission Guidelines for Virology

Sample selection, as well as proper collecting and handling of samples, is of critical importance prior to the submission of samples for virology.

Please consider the following points.

1. Appropriate samples should be selected after the probable diagnoses have been considered. See manual for a listing of suggested specimens for each virus. If your virus of choice is not listed in this guide, please call the ADDL for instructions.

2. Swabs, fecals, or tissue samples taken as aseptically as possible from animals in the early stages of clinical disease, are best. Most viral infections are cleared approximately 5 days after onset of signs.

3. Postmortem samples should be taken immediately after the animal has died. Organ tissues, to be evaluated separately, should be taken aseptically and packaged individually in plastic bags. Segments of gastrointestinal tract (2-4" segments) should be submitted intact (unopened) and tied off with string or twine. Intestines or contents should not be placed in the same plastic bag as organ tissues. Additional tissue is required when more than one examination (e.g., VI, FATS, EM) is requested.

4. Selection of the area of larger organs, such as lung, liver, and brain, is often important. In tissue with marked changes, higher levels of virus are generally found at the active edge of a lesion. Necrotic centers of lesions or areas of normal tissue are often non-productive specimens for isolation.

5. Fecals preserved with one (1) part formalin to nine (9) parts feces are examined by electron microscopy for viral particles. Fresh, chilled fecals can be processed for viral isolation and/or electron microscopy. Do not freeze materials to be examined by electron microscopy.

6. Samples should arrive in the laboratory as quickly as possible in a chilled state (32-34°F). Insulated containers with ice packs are suitable for this purpose. Avoid fluctuating temperatures and slow freezing. The level of virus in tissue (when it arrives at the laboratory) is always less than at the time of collection.

7. Pertinent information concerning history, premise ID, herd size, owner's name, numbers and ages of animals involved, vaccinations, etc., should accompany the samples. When possible, be specific as to the types of viral examinations being requested to avoid unnecessary charges.

8. Please notify the Virology Laboratory 24-48 hours prior to submitting large numbers (>20) simultaneously.

Submitting Ear Notches for Persistently Infected Bovine Viral Diarrhea (BVD)

Bovine viral diarrhea virus (BVDV) belongs to the family Flaviviridae, the genus pestiviruses. There are several members of the genus and two types of BVD viruses. All of them are highly infectious and of economic importance to the livestock industry. If a fetus becomes infected *in utero* with BVD virus in the early stages of pregnancy, the newborn calf will become persistently infected (PI) without immune response to this virus for the rest of its life and will shed BVD virus in the herd, infecting other animals. If a PI animal is not identified in the herd, it will be a source of infection. Elimination of BVD from the herd requires removing PI animals.

There are several assays available to identify PI animals: Polymerase chain reaction (PCR), virus isolation (VI), immunohistochemistry (IHC), and antigen-capturing (Agc) ELISA. The AgcELISA is a rapid diagnostic tool that can identify PI animals in the herd. Serum and ear notch samples are suitable for PI animal testing by AgcELISA for BVD viral antigen. To provide optimum service on this assay, the following guidelines should be followed when sending ear notch samples to ADDL.

1. Ear notches should be taken with a sharp ear notching tool suitable for adult swine.
    - Baby pig ear notches, punches, and other cutting and punching tools are NOT recommended; the sample they provide is too small for an accurate test.
    - Dull notches can damage animals and samples are not sufficient.

2. Ear notches must be fresh and the samples must be approximately 1cm x 1cm (3/4 x 3/4 inches) in size.
    - Avoid testing scabby or frostbitten ears.
    - DO NOT put samples in formalin.
    - Samples should be submitted fresh and chilled. For short-term storage (1-2 days), samples can be refrigerated at 39°F; for long-term storage, samples can be stored at –4°F or colder.

3. Package individually in snap cap tubes (12x75 mm). These can be obtained from the following vendors: Fisher Scientific at <www.fishersci.com> (catalog #14-959-2A) or VWR at <www.vsrsp.com> (catalog #60818-419).
    - DO NOT use Whirl Pak bags to submit samples.
    - Number tubes 1,2,3, etc., with animal identification to match information on accession form. Numbers should be clearly marked and legible.
    - If submitting more than 200 ear notches, please call the ADDL prior to submission to allow for the quickest possible processing and reporting of samples.

4. A PCR test for pools of up to 25 ear notch or serum samples is available. If the pooled sample is positive, ear notches or serum samples in that pool will be re-tested individually by antigen capture ELISA. Submit individual fresh samples in 5 ml sterile snap cap tubes, labeled with the animal identification, and a completed ADDL submission form. Specify that you are requesting the pooled BVD PCR.

    Samples will be pooled at ADDL.

    It is strongly recommended that ear notchers be disinfected in 10% bleach after each sample is collected. Do not vaccinate or tattoo animals at the same time ear notches are taken.

Agent	Specimen	EM	FA	VI	AgC ELISA	Comments
Adenovirus	Feces, lung, intestine	X		X		
Bluetongue Virus	Lung, spleen, heparinized blood		X	X		See also Serology section
Bovine Parainfluenza Virus (PI3)	-Fetal lung, spleen, kidney, liver -Nasal secretions, trachea		X	X X		
Bovine Parvovirus	-Feces -Fetal organs -Small intestine (6" segents)	X	 X X	 X		
Bovine Respiratory Syncytial Virus (BRSV)	Lung (affected portions), trachea		X			
Bovine Rotavirus	-Middle/lower intestine (tied off–6" segments) -Feces (early onset)	 X	X		 X	 ELISA available for Group A rotavirus
Bovine Viral Diarrhea Virus (BVDV)	-Buffy coat (EDTA blood) -Ear notch -Fetal organs -Lung, intestine, spleen, lesions -Serum -Swabs from lesions		 X X	X X X X	X X X	Requires 8-10 ml unclotted blood For detection of PI animals—PCR for genotyping available See p. 45 for specific instructions on ear notch testing See also Serology section
Breda virus	Feces	X				
Canine adenovirus (1,2)	Liver, kidney, urine, lung		X	X		
Canine coronavirus	-Feces -Small intestine	X	 X			
Canine distemper virus	-Brain, lung, spleen, bladder, liver -Conjunctival smears -EDTA blood (buffy coat)		X X	X X		
Canine herpesvirus	-Liver, lung, kidney -Saliva, nasal or vaginal secretions urine		X	X X		
Canine influenza virus	Throat swab, lung, nasal swab			X	X	PCR for group A influenza virus also available
Canine parainfluenza virus	-Lung -Nasal/throat swabs		X	X X		
Canine parvovirus	-Feces (early onset) -Intestinal contents -Tongue, small intestine, spleen -Heart—puppies < 10 wks	X X	 X X	X X X X	X X	
Canine rotavirus	Feces, small intestine	X	X		X	ELISA for Group A rotavirus
Encephalomyocarditis	-Brain, heart, lung, fetal organs -Thoracic fluid		X	X X		
Enterovirus	Brain, spinal cord			X		See also Serology section
Epizootic Hemorrhagic disease	Lung, spleen, heparinized blood		X	X		See also Serology section
Equine adenovirus	-Mandibular salivary gland, lung, liver, spleen, kidney -Nasal/ocular swab		X	X X		
Equine arteritis virus	Nasal swab, EDTA blood, fetal organs, bronchial lymph node, spleen, lung			X		See also Serology section
Equine Coital Exanthema (EHV3)	Swabs from lesions/vaginal swabs			X		

Agent	Specimen	EM	FA	VI	ELISA	Comments
Equine encephalitis (EEE/WEE/VEE)	Brain					Sent to NVSL
Equine herpesvirus 1,4	-Fetal lung, liver, kidney -Nasal/gingival swabs -Tracheal washings, lung, trachea, brain, spinal cord		X X	X X X		See also Serology section.
Equine infectious anemia virus	Serum					See Serology section
Equine influenza virus	-Lower respiratory tract, lung -Nasopharyngeal swabs		X	 X	X X	PCR available—See Molecular Diagnostics section
Equine rhinopneumonitis (EHV-4)	-Lung, trachea, brain, spinal cord -Tracheal washings		X	X X		
Feline calicivirus	Lung, gingival/nasal swabs, tongue			X		
Feline enteric coronavirus	-Feces -Small intestine-segments of distal duodenum, ileum, jejunum	X	 X			
Feline Immunodeficiency Virus (FIV)	Serum				X	Kit detects antibody
Feline Infectious Peritonitis virus (FIP)	Lung, liver, brain, kidney, effusion		X			
Feline Leukemia Virus (FLV)	Serum				X	Kit detects FLV antigen
Feline Panleukopenia virus	Spleen, lung, distal jejunum, ileum, fetal tissues		X	X		
Feline Rhinotracheitis	-Lung, liver, spleen -Nasal, ocular or pharyngeal swabs		X	X X		
Feline Rotavirus	-Feces -Intestine	X	 X		X	AgcELISA for Group A rotavirus
Hemagluttinating Encephalomyelitis virus (HEV)	-Brain, tonsil, lung, intestine -Feces	 X	X	X		
Classical Swine Fever virus	Tonsil, spleen, mandibular lymph node					PCR available—See Molecular Diagnostics Section
Infectious Bovine Rhinotracheitis virus (IBR)	-Fetus, lung, liver -Lung, trachea, bronchial lymph nodes		X	X		See also Serology section.
Infectious Canine Hepatitis						See Canine Adenovirus
Ovine Contagious Ecthyma (ORF)	Biopsy or lesions, scabs	X				Presumptive for Pox Virus
Papular Stomatitis	Lesions	X	X	X		
Porcine Adenovirus	-Feces -Intestine, lung, lymph node	X	 X	 X		
Porcine circovirus	Lymph nodes, tonsil, spleen, kidney, lung	X	X	X		-Porcine circovirus sequence analysis of ORF2 region -Porcine circovirus full genome sequence -PCR available—See Molecular Diagnostics section -See also Serology section
Porcine Parvovirus	Fetal tissues, mummified fetus		X	X		See also Serology section
Porcine Respiratory and Reproductive Syndrome virus (PRRSV)	Serum, lung, spleen, tonsil, lymph node		X	X		-Virus sequence analysis of ORF 5 region -PCR available—See Molecular Diagnostics section -See also Serology section

Agent	Specimen	EM	FA	VI	ELISA	Comments
Porcine Respiratory Corona Virus	-Lung -Nasal swabs		X	X X		-A positive result indicates either PRCV or TGE. Differentiation by PCR is available. See Molecular Diagnostics Section -ELISA available—See Serology section
Pseudocowpox	Skin lesions	X		X		
Pseudorabies virus(porcine)	-Brain stem, spinal cord, tonsil, Lung -Fetal spleen, liver, lung		X X	X X		See also Serology section
Pseudorabies (other species)	Brain stem, spinal cord		X	X		See also Serology section
Rabies virus	Brain stem, cerebellum					Sent to Indiana State Department of Health, Indianapolis
Rotavirus	-Feces -Upper, middle, lower intestine (6" segments-tied off)	X	X		X	-Do not open intestines. -See also Serology section
Swine influenza virus group A	-Nasal swabs, thoracic fluid -Lung, fetal organs		X	X X	X	-Swabs made of rayon or synthetic material are preferred over cotton. Viral isolates can be typed by PCR to differentiate H1N1, H1N2, H3N1 H3N2. See Molecular Diagnostics section -Virus Group A HA gene can be sequenced. -Serology assays are available to detect antibody to H1N1 and H3N2 virus. See Serology section.
Teschan, Tatfan (Enterovirus)	Brain, spinal cord			X		See also Serology section.
Transmissible Gastroenteritis virus (TGE)	-Feces -Upper, middle, lower Intestines, 6" segments	X	X	X		-PCR available. See Molecular Diagnostics section -See also Serology section.