

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. 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. Samples received after 2PM will be tested the following day.**
 - 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 C.E. 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).

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. Samples run at 1:50, 1:100, 1:200 dilutions. Titers: 1:50 - Suspicious ≥1:100 – Positive
		Refrigerated	TA	M,Tu,W,F	
Calicivirus	Serum	Refrigerated	IFA	M-F	IgG, IgM determination. Samples run at 1:10, 1:50, 1:250, 1:1250 dilutions..

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
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..
Parvovirus	Intestine (jejunum, ileum), spleen, mesenteric 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, 1:250, and 1:1250 dilutions.
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	IFA	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 Herpesvirus	Nasal swab, conjunctival swab, tonsil, trachea, lung	Refrigerated	FA	M-F	Reported as positive or negative
	Serum	Refrigerated	IFA	M-F	Sample are tested for IgG titers at 1:10, 1:50, 1:250 and 1:1250 dilutions.
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	
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, grippityphosa, 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. Reported as positive or negative
	Serum	Refrigerated	LA		Reported as positive or negative
	Serum	Refrigerated	ELISA		
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
Bovine Viral Diarrhea Persistent Infection (BVDPI)	Serum, ear notch	Refrigerated	ELISA	M-F	Instructions for ear notch submission
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	Thu	

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