

SAMPLE SUBMISSION FOR DISEASE INVESTIGATION

Where there is any doubt as to what samples to collect and how to transport them contact LABORATORY FIRST!

Clinical Pathology

Hematology:

EDTA is the preferred anticoagulant for most hematologic examinations requiring whole blood. However, differential leukocyte count and blood parasite examinations require unstained, thin blood films (*air - dried*) prepared from freshly collected samples. Provide duplicate blood films packaged to prevent breakage and to maintain dryness. Smears should be protected against moisture, insects, and fixatives, especially formalin or formalin fumes, since any of these will destroy or alter erythrocyte morphology. The problems we most often encounter are smears which are too thick (*no feathered edge*), have been fixed (*usually mailed in the same container as histology specimens*), are too degenerated (*smears not made soon enough after drawing blood*) or are inadequately stained. Prepare bone marrow aspirates in triplicate as described for blood films.

Cytology:

Cytologic examinations require 3 – 5 smears or touch impressions of varying thickness. Air dry rapidly. Package to prevent breakage and maintain dryness. Include location from which sample was obtained and history.

Urinalysis:

Submit urine in a clear, sterile tube under refrigeration.

Blood Chemistries:

Serum should be removed from the clot within 2 hours and free of hemolysis. Heat and bacterial contamination deteriorate serum constituents. To prevent deterioration, freeze serum after collection and maintain frozen during shipment.

Serology

Collect blood samples in clear sterile tubes (*Vacutainer tubes-PREFERABLY*). Fill tubes 3/4 full and allow to stand at room temperature for a few hours to permit a solid clot to form and retract. It is recommended that the serum be removed and the sample refrigerated. If the serum is to be held, it should be frozen. Do not freeze whole blood or samples with the clot remaining.

Many serological procedures require using live tissue cultures. Hemolyzed, contaminated, or toxic samples cannot be used.

Specimen:

Fluids should be sent under refrigeration. Smears should be unstained, air dried, and unfixed. **DO NOT** use any form of fixative. **DO NOT** add oil or any other substance to ease smearing of aspirates since such materials interfere with the staining procedure. Be specific in requesting tests, e.g., kidney function, liver function, electrolytes, or ask for specific tests which are necessary for diagnosis. If only a small quantity of serum is submitted, ask for the tests in order of preference.

Abortion Samples to Collect

Bacteriology (*Do NOT Freeze*):

Lung, liver, kidney, placenta (*Whirl-Pack bag*) cotyledon / caruncle (*placenta in separate bag*)

Fetal stomach contents (*Red Top Vacutainer tube*)

Cervical mucus (*Red Top Vacutainer tube*)

Vaginal swab Culturette

Serology:

Fetal heart blood or fetal thoracic fluid (*Red Top Vacutainer tub*)

Dam's sera at time of abortion and 10 – 21 days later (*Red Top Vacutainer tube*)

Histopathology:

Placenta (*chorioallantois and amnion*) , liver, kidney, adrenal, small intestine, lung, heart, thymus, brain (*Buffered formalin jar*)

Bacteriology

Used correctly, microbiologic cultures can identify etiologic agent(s) and so contribute key information towards a diagnosis. However, improperly used microbiologic cultures can identify contaminants or overgrowing organisms and lead to erroneous diagnoses. Actual etiologic agents can be lost due to improper transport medium, improper transport environment, or improper preservative techniques. The value of microbiologic culture depends to a considerable degree on the care and skill with which cultures are taken, stored, and shipped to the laboratory.

We offer the following guidelines to optimize these procedures:

- 1.** Samples should be collected aseptically and placed in sterile plastic bags or heat sterilized containers. Seal tightly. Do not use chemically disinfected containers, or plastic gloves or sleeves.
- 2.** Label all submissions with the location (*tissue*) and species of origin. The same bacterial species may be highly significant or a meaningless contaminant, depending on the tissue and/or species from which the sample was obtained. Also, depending on the tissue/species or origin, different culture requirements may be necessary to isolate and identify specific pathogens.
- 3.** Always specify the tests you want done, and the pathogens you suspect, particularly in the case of specimens with normal bacterial flora (*feces, intestinal contents, skin, or oral mucus membranes*). If we don't know what you are looking for, we may not inoculate the proper media to find it.
- 4,** It is best to collect other samples before opening the gastrointestinal tract. Tissue samples (*lung, liver, spleen, kidney, etc.*) should be 2 cm or larger to allow surface searing in the laboratory to reduce contaminants. Fecal

samples should not be submitted in stoppered tubes, as fermentation will dislodge the stoppers.

5. Place each sample in a separate container. If the intestine is to be cultured, tie off both ends of a segment and place in a separate container.

6. Fluids for culture (*i.e.*, *body cavities, pericardium, joints, etc.*) are best collected with a culturette or submitted in a sealed sterile tube or blood culture bottle. Fetal fluids (*thoracic or peritoneal fluids, heart blood*) to be examined for leptospiras should be submitted in a sealed sterile tube as soon as possible. Never submit fluids or other specimens in EDTA blood tubes, as EDTA is highly toxic to bacteria.

7. Milk samples should be submitted in screw-top tubes and frozen or placed on ice packs. Less than 1 ml is required for culture, and larger volumes are undesirable, if the samples are frozen. If an evaluation of somatic cell count is made, larger volumes (*10 ml*) of milk is required.

8. Specimens for isolation of anaerobic pathogens require special care. Anaerobic bacteria die in the presence of oxygen and should be shipped in an reduced container, such as anaerobic swab Cultures for *Clostridium* spp. in parenchymatous organs and intestines ordinarily provide little significant information concerning the cause of death if the samples are taken more than one hour after death.

9. Some specimens, such as porcine nasal swabs for *Bordetella* spp. isolation must be delivered to the laboratory within 12 hours of collection. Fastidious organisms such as *Campylobacter* spp. require special media for transport to the laboratory.

10. Keep specimens cold from the time they are collected until they arrive at the laboratory. Specimens should be shipped in insulated containers with a sufficient number of ice packs to last 48 hours. Specimens arriving in the laboratory in a decomposed state will not be processed. These tissues lead to meaningless or erroneous results.

11. For cases where bacteremia is suspected and blood culturing is requested, blood culture systems should be inoculated with the proper amount of blood collected aseptically. Single bottle blood culture systems are

recommended. Submit to the laboratory immediately in an insulated container with ice packs.

Mycology

- 1.** For dermatophyte cultures, the affected area of skin should be washed with 70% alcohol, scrapings taken from the active border areas and placed in a sterile container. The basal portion of several hairs should be plucked out with forceps and submitted as well.
- 2.** Exudates or tissues for culture should be collected aseptically, refrigerated, and sent to the laboratory on ice packs. Hair and skin scrapings for dermatophyte isolation should be shipped dry at room temperature in a paper envelope.
- 3.** Isolation and identification of some mycotic agents may require a minimum of 30 days.

Histopathology

Histopathology continues to be a powerful, yet inexpensive part of veterinary diagnostics.

- 1.** Specimens should represent typical lesions, including active margins and adjacent (*normal*) tissue, rather than lesion cores or curetted debris. Autolysis, freezing, mutilation (*forceps crushing or tearing*), or removal of small samples by electrocautery may make samples unsuitable for evaluation.
- 2.** Multiple specimens (*from different sites or types of lesions*) should be identified individually by size, suture tags, or separate containers. Samples should be no thicker than 0.5 cm, to allow adequate fixation. Brain and eyes are exceptions; they should be fixed whole.
- 3.** Nearly all diagnostic histopathology can begin with tissue fixed in 10% buffered neutral formalin.

Formula:

37-40% formaldehyde
100 ml distilled water
900 ml sodium phosphate, monobasic
4.0 gm sodium phosphate
6.5 gm dibasic

Fixative volume should be 10 times specimen volume. After 12 – 24 hours, specimens can be transferred to just enough formalin to keep them moist during shipment. There is no need to pay for transport of excess fixative.

Formalin will freeze at low temperature, damaging the tissues. Adding 1 ml of ethanol to each 9 ml of 10% formalin will prevent such freezing.

4. Wide-mouth plastic bottles or Whirl – Paks are preferred containers.

Do not use baby food jars. Narrow – mouth bottles often have to be broken or cut to release fixed tissues. **Do not send tissues in glass containers.** Anticipate rough handling during shipment, and package accordingly. Most bottle lids will leak; if in doubt, tape the lid. Label container(s) adequately (*owner, animal, veterinarian, site*). Interpretation of histopathologic findings often hinges on complete clinical histories.

5. Submit specimens from all major organs, including brain, if in doubt about which tissues to collect or if there are no gross lesions.

6. Fresh tissue, handled gently and fixed adequately in 10% buffered neutral formalin will yield excellent results. Some pathologists, however, have advocated using Bouin's fixative for endometrial and endocrine specimens. The advantages, in our opinion, of Bouin's do not outweigh the disadvantages of extra reagents and processing steps. Tissues should be fixed in Bouin's no longer than 18 hours, or they become hard and brittle. Specimens must be washed 4 – 6 hours in several changes of alcohol to remove any picric acid (*yellow*), then stored/shipped in 70% alcohol. Over fixation with Bouin's results in poor histologic staining.

7. Duplicate glass slides of specimens can be prepared for practitioner's use and files.

Parasitology

Fecal samples should be mailed in plastic bags or other water – tight containers. If the samples reach the laboratory in three days, no

preservatives need to be used. However, some of the ova may hatch during this time unless air is excluded from the container. The recommended method is to place the fecal sample in 10 – 15 volumes of 10% formalin (*the same concentration as for submitting histopathology samples*) or, if coccidia are suspected, place the feces in 2.5% potassium dichromate solution. If special procedures such as the Baermann technique to find *Strongyloides* spp. or lungworm larvae are needed, they should be requested. If examination for intestinal protozoa other than coccidia (*particularly Giardia spp. or amoebae*) is requested, the sample should be fixed and shipped using sodium acetate – acetic acid – formalin (*SAF*) fixative. The fixative also preserves helminth eggs. The SAF solution is prepared as follows:

Sodium acetate 1.5 g

Acetic acid, glacial 2.0 ml

Formaldehyde solution (40%) 4.0 ml

Water 92.5 ml

TOTAL 100.0 ml SAF fixative.

Mix one volume of feces with at least three volumes of SAF fixative. Shake container well to ensure complete dispersal of specimen.

Parasite Specimens:

Gross specimens require fixation and preservation prior to mailing. Trematodes and cestodes that are recovered at necropsy or passed in the feces should be placed in tap water, kept overnight in a refrigerator, and then fixed and stored in 10% formalin. Nematodes should be fixed, preferably in 70% ethanol, but can also be fixed in 10% formalin.

Most nematodes from domestic animals can be easily identified, but if nematodes are from exotic or wild animals, special fixation is needed. The best method is to heat 70% ethanol to about 60 degrees Celsius, drop the parasites into the hot alcohol one by one, and remove them as soon as they are fixed in an extended position. They should then be placed in fresh 70% alcohol for mailing.

Blood specimens for examination for microfilaria (*Knott's test*) should be fixed in at least 10 volumes of 2% formalin. Serum (*1 ml*) should be sent for the occult heartworm antigen test.

Ectoparasites should be fixed and submitted in 70% alcohol. Skin scrapings may be sent on slides if a coverslip is sealed to the slides with clear nail polish or similar material. Be sure there is enough oil under the coverslip so that the material will not dry out in transit. Send more than one slide if possible. In addition, submit a scraping from the same area the parasites were found. Take scrapings from the edges of lesions and try to squeeze the skin while scraping to bring mites to the surface. A good, deep scraping is indicated by the presence of red cells. Submit in 70% alcohol.

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SAMPLES TO BE SUBMITTED-DISEASE / DISCIPLINE WISE

| Sr. No. | Disease | Specimen | Discipline |
|----------------|--|--|---|
| 1 | Actinomycosis/ Actinobacillosis | abscess material fixed abscess lesion | Bacteriology Histology |
| 2 | Anthrax | Peripheral blood, Piece of Ear | Bacteriology |
| 3 | Black Leg /Malignant Edema | fresh skeletal muscle fixed skeletal muscle | Bacteriology Histology |
| 4 | Bluetongue | 10 ml heparinized blood (<i>Green top tubes</i>) Serum (<i>CF,SN,ELISA</i>) | Virology Serology |
| 5 | Border Disease (BD/BVD) | Dam: rectal, nasal swabs, whole blood (<i>heparinized preferred</i>) , serum Lamb: 1/2 fresh brain, spinal cord, CSF,serum 1/2 fixed brain, spinal cord | Virology Virology Histology |

| | | | |
|-----------|--|--|--|
| 6 | Brucellosis _____ Livestock _____ Ovine | heparinized blood Serum | Bacteriology Serology |
| 7 | Canine Distemper | fixed brain, lung, urinary bladder, stomach, liver, kidney | Histology |
| 8 | Caprine Contagious Arthritis/Encephalitis | fixed brain, synovial membrane, lung, mammary gland serum | Histology Serology |
| 9 | Coccidiosis | feces, intestine smears fixed duodenum, jejunum, ileum, and colon | Parasitology Histology |
| 10 | Colibacillosis _____ Mammalian _____ Avian | fresh duodenum, jejunum and ileum fixed duodenum, jejunum, ileum, colon fresh liver, air sac swab fixed liver, spleen, air sac | Bacteriology Histology Bacteriology Histology |
| 11 | Contagious Ecthyma <i>(sore mouth, ORF)</i> | fresh skin scrapings and crusts, vesicular fluid, serum fixed biopsy | Virology Histology |
| 12 | Dirofilariasis <i>(canine, feline Dirofilaria immitis infection)</i> | Whole Blood/in EDTA | Parasitology |
| 13 | Equine Infectious | fixed heart, liver, kidney, | Histology |

| | | | |
|-----------|------------------------|--|---|
| | Anemia | spleen serum | Serology |
| 14 | FMD | vesicular fluid, tongue lesion serum | Virology Serology |
| 15 | Johne's Disease | fresh feces, ileum, spiral colon, regional mesenteric lymph node fixed ileum, spiral colon, regional mesenteric lymph node serum milk | Bacteriology Histology Serology Serology |
| 16 | Mastitis | fresh milk; use proper sample container half full or less. | Bacteriology |
| 17 | PPR | Illium, Mesenteric Lymph nodes (MLN), tongue lesions fixed ileum, spiral colon, MLN | Virology Histology |
| 18 | Rabies | fresh brain | Virology |
| 19 | Salmonellosis | fresh lung, liver, jejunum, ileum, mesenteric lymph node, colon, colonic lymph node, kidney, spleen fixed lung, liver, duodenum, jejunum, ileum, colon, kidney, spleen, lymph nodes | Bacteriology Histology |

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|-----------|--|--|---------------------------|
| 20 | Semen Testing, Bovine Viral Isolation | 6 straws or equivalent per animal frozen in liquid nitrogen | Virology |
| 21 | Shipping Fever-(HS) <i>(Bovine)</i> | fresh lung, trachea, ocular swabs, nasal swabs, respiratory lymph nodes, spleen | Bacteriology |
| 22 | Strangles | abscess exudate | Bacteriology |
| 23 | Systemic Fungus | fresh involved organ fixed lung, liver, other involved organs | Bacteriology Histology |
| 24 | Trichomoniasis | uterine exudate, preputial scraping | Bacteriology |
| 25 | Tuberculosis | fresh involved tissue fixed lung, liver, other involved organs | Bacteriology Histology |
| 26 | Vesicular Stomatitis | fresh vesicular fluid, scrapings, biopsy, serum fixed biopsy of lesion | Virology Histology |