

BACTERIOLOGY SUBMISSIONS

AEROBIC SPECIMENS FROM NECROPSIED ANIMALS

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

MASTITIS MILK SPECIMENS

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

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

ANAEROBIC AND MICROAEROPHILIC SPECIMENS

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

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

MYCOLOGY (Fungal culture)

COLLECTION AND CARE OF SPECIMENS

1. Submit skin scrapings from the outer edges of a lesion and submit plucked (not cut) hairs.
2. Skin, hair, and nails should be shipped to the laboratory without refrigeration.

3. Submit internal organs or internal lesions suspected of fungal infection.
4. Internal specimens should be sent refrigerated (wet ice packs) and not frozen. Use whirl-paks and insulate.

RESULTS

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

MYCOLOGY NOTE:

Blastomyces, Histoplasmosis, and Coccidioides are highly pathogenic dimorphic fungi that pose a significant risk to laboratory personnel. These organisms produce numerous arthrospores which are readily disseminated in the culture process; therefore safety precautions in the laboratory preclude culture of these organisms at this time. These diseases are best diagnosed by serological methods, cytology or histopathology. These methods also provide a more rapid diagnosis than fungal culture.

USER'S GUIDE TO THE ANIMAL MICROBIOLOGY UNIT

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

TEST	SAMPLE
Abortion Screen	Placenta, fetal stomach contents, uterine contents, (includes culture ID for Trichomonas, Campylobacter, and Brucella)
Acid Fast Stain	Feces or intestine (for Johne's Mycobacterium paratuberculosis and Cryptosporidia).
Aerobic Culture	Fresh chilled tissue, urine, exudate, transtracheal wash
Anaerobic Culture	Fresh tissue, anaerobic culturettes, exudate
Antibiotic Sensitivity (disc diffusion, Vitek)	Performed on isolates recovered from specimens. Please request on accession form that you would like this test done.
Blood Culture	Blood submitted in blood culture bottle
Brucella abortus Culture	Fetal tissues, placenta, milk, lymph nodes
Brucella canis	Fetus, testicle, placenta, uncoagulated blood in a blood culture bottle, lymph nodes, vaginal discharge, milk, semen
Calf Scours	Feces or affected intestine
Campylobacter (Vibrio)	Preputial wash, vaginal fluid. CALL FOR PROTOCOL for protocol for submitting

Culture (bovine)	samples.
Campylobacteriosis	Affected intestine
Candida	Lesion, milk
Dermatomycosis	Lesion, hair, scales, fungal slants/trays
Dermatophilosis	Hair and scabs (please submit a good size sample)
Diarrhea/enteritis	Feces, affected intestine
Johne's (Mycobacterium paratuberculosis)	Feces (2 gram sample, Walnut-sized sample)
Listeriosis	Cerebellum, pons, medulla, fetus, uterine secretions
Lumpy Jaw (Actinomyces)	Exudate, lesion, sulfur granules
Mastitis (milk) culture	Milk submitted in whirl-paks or sterile tubes. Please NOTIFY the laboratory in advance if submitting more than 40 samples at a time.
Mycoplasma	Fresh chilled tissue, transtracheal wash, swab (may require 10-14 days for completion).
Paratuberculosis (Johne's).	Feces (2 gram sample, Walnut-sized sample)

Pinkeye (Moraxella bovis)	Culturette of affected eye. Sometimes difficult to isolate from normal flora.
Pneumonia	Lung (Please indicate if Mycoplasma, Hemophilus, or Rhodococcus is suspected.)
Salmonella	feces, feed, water, environmental samples
Streptococcus equi (Strangles)	Exudate from non-draining lesion
Trichomonas culture	Preputial wash, vaginal fluid, CALL FOR PROTOCOL before submitting samples

**PROTOCOL FOR TRICHOMONAS and BOVINE CAMPYLOBACTER
COLLECTION AND SUBMISSION IN CATTLE**

TRICHOMONAS

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

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

MATERIALS REQUIRED:

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

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

- Step 1 Use a separate pair of gloves and a separate collection device for each animal.
- Step 2 Clip the preputial hairs to about one-half inch and clean the preputial orifice.
- Step 3 If necessary; rinse out the preputial cavity with sterile saline (not water) to clean out mud and manure. This will help prevent contamination from non-pathogenic intestinal trichomonads and coliform bacteria.
- Step 4 Insert the sampling device (pipette inside tube) into the preputial opening to about the distal third of the preputial cavity. Advance the collecting pipette through and beyond the protecting tube to the preputial fornix.
- Step 5 Collect the sample by rapidly scraping the pipette back and forth in short strokes on the mucosa of the distal penis and fornix area while applying suction with a rubber bulb or syringe and massaging the glans penis through the sheath to move smegma into the pipette. Fifteen to thirty (15 – 30) strokes of the pipette are required to obtain an adequate sample.

Female Animals:

- Step 1 Use a separate pair of gloves and a separate collection device for each animal.
- Step 2 Clean debris from the vulva.

Step 3 Immobilize the cervix per rectum and insert the sampling device into the anterior third of the vagina.

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

The specimen should not be refrigerated or frozen.

Inoculation of InPouch™

Step 1 Remove the pouch from the bag, and manually express the liquid so that there is approximately 1 ml in the upper chamber.

Step 2 Tear the pouch open at the notch just above the closure. Open the pouch by pulling the closure tape's middle tabs apart.

Step 3 Bovine infusion pipette - Insert the specimen pipette tip into the liquid of the pouch's upper chamber and expel 1.0-1.5 ml of the sample into the pouch. If the collected material adheres to the wall of the pipette, rinse the pipette by flushing a small amount of the liquid medium back and forth into the pouch, minimizing the production of bubbles. This maintains an anaerobic environment.

If using a swab, insert the swab into the liquid of the pouch's upper chamber and press the tip of the swab between the fingers through the flexible walls of the pouch.

Step 5 Express the media into the lower chamber and roll the pouch down until the tape is at the top of the label. Fold the wire tape's end tabs to lock the roll. Write the animal ID on the label.

For Campylobacter:

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

Shipping Requirements:

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

¹BioMed Diagnostics, Inc.

1388 Antelope Road

PO Box 2366

White City, Oregon 97503

1(800) 964-6466

www.biomeddiagnostics.com

InPouchTMTF Test – Bovine Cat # 11-1003 100 tests

InPouchTMTF Test – Bovine Cat # 11-1001 20 tests

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

