



Animal Health  
Fact Sheet



# PROTOCOL FOR TRICHOMONAS DIAGNOSIS IN CATTLE FOR UTAH

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## DIAGNOSIS OF TRICHOMONAS

(Includes Regulatory Amendments effective 3/03/2004.)

Diagnosis of trichomoniasis is made when trichomonad organisms are observed in the smegma or preputial flush samples of bulls, or the uterine/vaginal fluid of cows. The organisms may be observed by direct microscopic examination of the fresh samples or by examination of culture media inoculated with infected material.

Because of the potential for contamination of a sample with the “fecal trich” organism, an owner of a positive bull, 16 months of age or younger, may request submission of the positive sample to an approved laboratory for PCR confirmation. The positive sample must arrive at the laboratory within 48 hours of being found positive. A sample that is inconclusive will be considered positive. A sample determined to be negative to *T. foetus* by PCR will be considered negative. But the bull must also be subsequently tested negative by culture, prior to being offered for sale and no sooner than 30 days after the PCR test.

## SAMPLE COLLECTION

1. The preferred sample is from the glans penis. This can be obtained by using a sterile insemination pipette and performing a vigorous back and forth scraping motion along the glans while applying negative pressure with an attached 10 ml syringe. A separate pipette and syringe should be used for each animal.
2. A current official Trich tag should be placed in the right ear of each bull at the time of sample collection.
3. The preferred sample from the female is the cervical mucous or uterine secretions. These samples can be collected by applying negative pressure with a syringe attached to a sterile insemination pipette, while the pipette is positioned within the open cervix or positioned to collect fluid from the vaginal floor.

## MEDIA

(The listed media are currently the only officially recognized media for the culturing of bovine trichomoniasis organisms in the State of Utah.)

1. **InPouch TF Pouch:** The pouch is a commercially prepared and packaged proprietary media. The pouches are available from Bio-Med Diagnostics Inc., 1388 Antelope Road, White

City, OR 97503-1619. CALL TOLL FREE: 800-964-6466 Telephone: 541-830-3000 Fax: 541-830-3001

**2. Modified Diamond's Media:** The Modified Diamond's Media is a semi-solid media that is specially prepared at the Utah Veterinary Diagnostic Laboratory at Logan (435-797-1895) and at the Central Utah Branch Laboratory in Nephi (435-623-1548).

The directions for use of these two media will be described separately.

## **MEDIA INOCULATION, CULTURE AND MICROSCOPIC EVALUATION**

### **1. InPouch TF:**

Inoculate the sample into the small upper chamber of the pouch, flush out the pipette, and squeeze or "squeegee" the liquid down into the lower chamber. Carefully express air bubbles out of the lower chamber to maintain the anaerobic environment. Roll the top of the plastic pouch down to the top of the lower chamber and fold the wire strips across to hold and seal it. **Don't stir or mix the content and keep the packet upright at all times once the lower chamber is inoculated.**

**Shipping:** In-Pouch TF media can be transported by commercial carrier, but this must be by overnight express/one-day delivery. If the pouches are to be shipped, the samples should be left in the upper chamber and not pressed into the lower chamber until they arrive at the lab. This allows them to be handled freely but then once at the lab, the inoculation of the lower chamber can be completed and the pouch maintained upright from then on. Be sure the lab will be open to receive samples.

**Handling and Care of Pouches:** The handling and/or shipping of the inoculated media samples is one of the most critical steps in Trichomoniasis diagnosis. The inoculated media should be kept at 65° F to 75° F until it is incubated. It is especially important to avoid overheating or freezing. Ship the inoculated pouches in insulated containers (**no ice**) that will protect the samples from extreme temperatures. Trichomonads are very susceptible to either freezing or overheating. It is important to arrange shipping so the samples arrive at the laboratory or clinic that will perform the testing within 30 hours of collection. Only those samples which are received at a certified diagnostic facility within 30 hours from time of collection will be considered as conforming to requirements for a valid test.

**Culture:** Specimens arriving in the InPouch TF by shipping will need to be inoculated from the upper to the lower chamber and then put into the incubator. Pouches are incubated vertically (upright) at 37° C and examined daily until positive growth and confirmation occurs or until they have remained negative for 96 hours.

**Microscopic examination:** Some veterinarians feel they can observe better for the trich organism by not using the viewing frame that is provided with the pouches. Carefully place the pouch on the stage, with as little disturbance of contents as possible. Focus the microscope on the crystals or debris present in the bottom of the pouch media, then move the pouch to observe other areas. Trichomonads generally will be found slightly above the bottom border. If the viewing frame is used, place the raised platform of the open viewing frame on the bottom of the lower pouch chamber, while it is in a vertical position. Close and lock the frame over the pouch and place the pouch, with frame, on the microscope under a 10x objective (100 power) and examine for typical motile organisms.

### **2. Modified Diamond's Media (MDM) (Tube Media):**

**Flushing the AI pipette:** Saline or Lactated Ringer's Solution may be used to flush the smegma or mucous from the AI pipette. Place 1-2 ml of either solution into a small test tube prior to collection of samples. After collecting a sample in the pipette, draw the solution

from the test tube up and down in the AI pipette to clean it out. Cap and label the tube to identify the animal sampled. These samples should be transferred onto the MDM (tube media) within 1-2 hours after collection (maximum of 6 hrs). They can be hand-carried to the lab and transferred there, IF that can be done within 6 hours. Keep the samples out of direct sunlight and away from excessive heat or cold (maintain at 65-75 degrees F). To transfer the saline and sample to the culture media, tap the sample tube to mix the smegma and solution, uncap both tubes, tip both at an angle and pour the sample gently down the inside of the MDM tube. Cap and identify the culture tube and place in the incubator.

The collected sample may be carefully inoculated directly onto the tubes of Modified Diamond's Semisolid Media. **Don't** put the AI pipette way down into the media and **don't** squirt the pipette contents into the tube. After collecting the smegma sample, carefully draw 1-2 ml of media into the AI pipette to flush the pipette. Flush it back and forth in the pipette and then carefully layer it back onto the top of the media. This care in layering is very important since the trichomonads need to migrate to the bottom away from other contaminating organisms. Mixing the media or inverting the tube will allow more bacteria to grow and inhibit growth of the trich organism. Identify and cap the tubes.

**Care of tube media:** Avoid shaking the tubes and do **NOT** invert them.

**Shipping and handling:** Tube media must be **hand delivered** to the laboratory. It cannot be sent by commercial carrier as the tubes must not be inverted.

**Culture:** Tube media that has been inoculated is put into the incubator, incubated vertically (upright) at 37° C (98-99 F), and examined daily until positive growth and confirmation occurs or until they have remained negative for 96 hours. Samples remaining negative through 96 hours are reported as negative.

**Microscopic examination:** A small portion of the media is removed from near the bottom of the tube (¼") with a long (9"), sterile pasteur pipette and a drop is placed on a clean glass slide. The slide is placed on the microscope under a 10X objective (100 power) and examined for typical motile organisms. Several samples can be placed on the same slide, but care must be taken to keep them properly identified. Be sure to use a new pipette for each tube. It is better to not use a coverslip for viewing.

## **TIMING OF CULTURE EXAMINATIONS**

(For either media system)

Samples should be closely examined for growth at 24 hour intervals for 96 hours. The results are recorded on the Official "Trichomonas Test and Report Form." Record the date of the reading at the top of the column above "Readings" then record the results for each sample in the column for that day's reading. The final results are recorded for day 4, or earlier for those samples on the test form that have already been found positive. (**Note: If you have positives on the first reading, you may want to call the owner and report the positives prior to Day 4).**

Upon completion of testing, the laboratory performing the test fills out the summary information, records the Laboratory name and address, and the laboratory supervisor or principal technician signs the forms. The forms are then forwarded according to the distribution list at the bottom of the form. The forms should be completed in their entirety, since they are a legal document.

## **INTERPRETATION OF RESULTS**

**POSITIVE:** A bull is considered positive if Trichomonas organisms are identified when cultured by the examining veterinarian or laboratory. An owner may have the option to request submission of the positive sample to an approved reference laboratory for confirmation by Polymerase Chain Reaction (PCR). As prerequisites to exercising this option, the bull must be 16 months of age or younger and the sample must arrive at the laboratory within 48 hours of being

found positive. A sample determined by PCR not to be T. foetus will be considered negative. A sample found to be inconclusive will be considered positive. A bull determined to be negative for T. foetus by PCR must be subsequently tested negative by culture prior to being offered for sale and no sooner than one month after the PCR.

**NEGATIVE:** A sample is considered negative when **no** viable, motile trichomonads are observed in the culture media during any of the readings.

## **REPORTING AND HANDLING POSITIVE BULLS**

All bulls testing positive for Trichomoniasis must be reported immediately to 1) the owner, and 2) the State Veterinarian by the veterinarian performing the test. The owner shall be required to notify the administrators of the common grazing allotment and any neighboring (contiguous) cattlemen within ten days following such notification by his veterinarian or laboratory.

All bulls which test positive to Trichomoniasis must be sent by direct movement within 14 days, to: 1) slaughter at an approved slaughter facility, or 2) to a qualified feedlot for finish feeding and slaughter, or 3) to an approved auction market for sale to one of the above facilities. Such bulls must move only when accompanied by a VS 1-27 Form issued by the testing veterinarian or other regulatory official. Positive bulls entering a qualified feedlot, or approved auction market shall be identified with a lazy V brand on the left side of the tail, indicating that the bull is infected with the venereal disease, Trichomoniasis.

## **SAMPLE DISPOSAL**

1. In accordance with the EPA and OSHA requirements for disposal of biological wastes, all trichomonas samples should be inactivated before disposal. This is best accomplished by autoclaving the tubes or pouches prior to discarding. If an autoclave is not available or if autoclaving is not practical, inactivate the tubes by adding Clorox, Nolvasan or some other disinfectant to the tubes or pouches and shaking vigorously prior to disposal.

2. All of the tubes or pouches should be discarded at the end of the 4-day incubation period and **all** should be inactivated regardless of whether the final results were positive or negative.

To obtain a copy of the official current regulation on trichomoniasis, request a written copy from them or go to the Utah Department of Agriculture and Food website at <http://ag.utah.gov/animind/ahealth.html>

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