



Protocol for Trichomonas Diagnosis in Utah Cattle

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Diagnosis of Trichomonas

This information includes Regulatory Amendments effective October 2009 (Utah R58-21).

Trichomoniasis, caused by the protozoan *Tritrichomonas foetus*, is a serious reproductive (venereal) disease found in some Utah cattle herds. Diagnosis of trichomoniasis is made when trichomonad organisms are observed in the smegma or preputial flush samples of bulls, or the uterine/vaginal fluids 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 contamination of a sample with fecal trichomonads, owners of a positive bull, regardless of age, may request submission of the positive culture sample to an approved laboratory for polymerase chain reaction (PCR) confirmation. The positive sample must immediately be frozen and shipped on ice to the Utah Veterinary Diagnostic Laboratory (UVDL) (950 East 1400 North, Logan, UT 84341) such that arrival can be guaranteed to happen when the laboratory is open and the sample remains frozen (i.e., overnight delivery).

Once frozen, the sample can remain in the freezer until delivery is arranged. Call the UVDL (435-797-1895) to ensure delivery and alert them of incoming samples. A sample that is inconclusive will be considered positive. A sample determined to be negative to *T. foetus* by PCR will be considered negative.

Sample Collection

1. With bulls, the preferred sample location is the glans penis. The glans penis is the area between the sheath and penis. Any hanging fecal balls or long hair at the preputial opening should be trimmed to minimize sample contamination. After insertion in the preputial opening, samples are obtained using a sterile insemination pipette (e.g., 25" length with 0.130" I.D. and 0.196" O.D.) and performing a vigorous, back and forth, scraping motion along the glans while applying negative pressure with an attached 10 or 20 ml syringe. The package insert for the pouch recommends a 20 ml syringe. **A separate sterile 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 under the direction of a certified veterinarian. Official Trich tags can be obtained by contacting the Utah Department of Agriculture and Food (UDAF), 350 N Redwood Rd, PO Box 146500, Salt Lake City, UT 84114-6500. Telephone: 801-538-7161; Fax: 801-538-7169.
3. The preferred sample from the female is the cervical mucus 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 is currently the only officially recognized media for the culturing of bovine trichomoniasis organisms in the State of Utah. Please note that Modified Diamond's Media (MDM) is no longer officially recognized by the State of Utah.

1. **InPouch™ TF Pouch:** The pouch is 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.

The directions for use of this media will be described separately below.

Media Inoculation, Culture and Microscopic Evaluation

InPouch™ TF: (please read the manufacturer's package insert)

While holding the pouch upright, tear off the upper edge where marked to allow access to the upper chamber. If some fluid from the lower chamber is not in the upper chamber, gently apply pressure to the lower chamber to transfer media from bottom to top. Inoculate the sample into the small upper chamber of the pouch. Flush out the pipette by drawing up a few milliliters of pouch solution into the pipette, repeating as necessary to sufficiently inoculate the upper chamber. The inoculated fluid in the upper chamber should be squeezed or "squeezed" into the lower chamber being careful to apply just enough pressure to evacuate the upper chamber without rupturing the pouch itself. 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. **Do not stir or mix the contents and keep the packet upright at all times once the lower chamber is inoculated.**

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 between 65°F and 90°F until it is incubated. It is especially important to avoid overheating or freezing. Handle the inoculated pouches in insulated containers that will protect the samples from extreme temperatures (hot or cold).

Trichomonads are very susceptible to either freezing or overheating. With the majority of Utah bulls being sampled during the winter season, cold temperatures are the greatest risk. Be sure to utilize a storage and transportation system between the farm and clinic that can ensure samples are kept above 65°F. Samples, regardless of evaluation technique used (i.e., culture or PCR), should be incubated as soon as possible at 98.6°F (37°C). Those samples evaluated via culture should be incubated for 96 hours (4 days) and observed microscopically every 24 hours during this period. Samples being shipped to an approved reference laboratory (i.e., UVDL) for culture evaluation should be shipped in an insulated container to ensure temperature extremes do not affect samples. If ambient shipping temperatures are such (below 65°F) that it is determined the samples will be subjected to cold temperatures, warming packs should be placed in the container to allow shipment with the optimum temperature zone. Samples being sent to the UVDL for PCR should be incubated for 24 hours at the veterinary clinic, frozen solid in the pouch by the referring veterinarian's staff, then shipped on ice as described previously. It is important to arrange shipping so the samples arrive to the laboratory in a frozen state. Samples lost in shipping, or that arrive thawed and warm, may be classified as unacceptable for testing and not valid.

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.

Shipping: In-Pouch TF media can be transported by commercial carrier, but this must be overnight express/one-day delivery. Samples need to be shipped upright. Be sure the lab will be open to receive samples.

Timing of Culture Examinations

Samples should be closely examined for growth at 24 hour intervals for 96 hours (4 days). 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 the 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 has the option to request submission of the positive sample to an approved reference laboratory for confirmation by PCR. A sample determined by PCR not to be *T. foetus* will be considered negative. A sample found to be inconclusive will be considered positive.

Negative: A sample is considered negative when **no** viable, motile, trichomonad organisms are observed in the culture media during any of the readings. Or, with PCR, no genetic material is identified during amplification and testing.

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 or laboratory 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 their veterinarian or laboratory.

All bulls which test positive to Trichomoniasis must be sent directly (within 14 days) to either slaughter at an approved facility, or to a qualified feedlot for finish feeding and slaughter, or to an approved auction market for sale to one of the above facilities. Such bulls must

move only when accompanied by a VS Form 1-27 (Permit for Movement of Restricted Animals) issued by an accredited 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 head, 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 pouches prior to discarding. If an autoclave is not available or if autoclaving is not practical, inactivate the pouches by adding sodium hypochlorite (Clorox®), chlorhexidine diacetate (Nolvasan®), or some other disinfectant 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 current regulation on trichomoniasis, request a written copy from UDAF, or by viewing the URL:
<http://ag.utah.gov/divisions/animal/health/index.html> .
The Utah Trich rule (R58-21) can be found at
<http://www.rules.utah.gov/publicat/code/r058/r058-021.htm>.

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This publication is issued in furtherance of Cooperative Extension work, acts of May 8 and June 30, 1914, in cooperation with the U.S. Department of Agriculture, Noelle E. Cockett, Vice President for Extension and Agriculture, Utah State University.