



Protocol for Trichomonas Diagnosis in Utah Cattle

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Diagnosis of Trichomonas

This information includes Regulatory Amendments effective May 1, 2012 (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 detected in the smegma or preputial flush samples of bulls, or the uterine/vaginal fluids of cows. Because of the potential contamination of a sample with fecal trichomonads, the use of an approved polymerase chain reaction (PCR) test differentiates between the two. Frozen samples are to be 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. 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, 350 N Redwood Road, SLC, UT 84114-6500 (801-538-7164). The color of the tag changes each year and markings correspond to the year and state (Figure 1).



Figure 1. Official Utah Trichomoniasis Tag. The number 12 represents the year (2012), the 87 refers to the state (Utah), and the large black number can be ordered in sequence to identify the animal.

- 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 **InPouch™ TF Pouch** 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. The pouch is a commercially prepared and packaged proprietary media. 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, Incubation, and Shipment for PCR Testing

While holding the InPouch™ TF 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. Please read the manufacturer's package insert for additional instructions and recommendations.**

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 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). Trichomonad organisms are very susceptible to either freezing or overheating. Samples should be incubated as soon as possible at 98.6°F (37°C) for 24 hours. Pouches should be frozen directly after the incubation period. Frozen samples should be shipped in an insulated container to ensure samples remain frozen. It is important to arrange shipping so the samples arrive at 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:

This method of detection of trichomonads is no longer an accepted definitive regulatory diagnostic test in Utah and bulls may not be classified based on culture examination.

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.



Figure 2. InPouch (image from Bio-Med Diagnostics)

Interpretation of Results

Positive: A bull is considered positive if Trichomonas organisms' genetic material is identified by Polymerase Chain Reaction (PCR). A PCR test that does not detect *T. foetus* will be considered negative. A sample found to be inconclusive will be considered positive.

Negative: A sample is considered negative when no genetic material is identified during amplification and testing by PCR.

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 and laboratory performing the test (respectively). The owner shall be required to notify the administrators of the common

grazing allotment and any neighboring (contiguous) cattlemen within 10 days following such notification by his or her 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, indicating that the bull is infected with the venereal disease, Trichomoniasis.

Bulls that are within a drug withdrawal period should not be sent to slaughter until the appropriate period has expired. In this case, it is the responsibility of the veterinarian to notify the State Veterinarian of this situation.

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 Trichomoniasis rule (R58-21) can be found at <http://www.rules.utah.gov/publicat/code/r058/r058-021.htm>

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