
STATE OF MONTANA

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Veterinarian Protocol for Trichomoniasis Testing in Bulls ***March 2023***

Diagnosis of trichomoniasis is made when trichomonad organisms are observed in the smegma or preputial flush samples of bulls or when the genetic material of the organism is detected using PCR testing. The organisms may be observed by direct microscopic examination of the fresh samples or by examination of culture media inoculated with infected material. Please be accurate and consistent in collection techniques and *pay close attention to the TF InPouch or TF-Transit directions.*

Official trich testing:

- Must be conducted by a deputy state veterinarian.
- Accepted tests include three weekly negative cultures, a single negative individual PCR, or a single negative pooled PCR. *Trich positive herds and trich adjacent/exposed herds are not eligible for pooled PCR testing.*
- Animal require a minimum of two weeks of sexual rest prior to sampling.
- For culture tests, no less than 7 days are required between each test, with no breeding activity during the intervals.
- Tests expire after 60 days or immediately upon commingling with female cattle.
- Includes tests for interstate movement, trich epizootic area testing, and required testing for animals grazing in common.

Identification:

Official individual identification should be applied **at the time of initial sampling**. You may use either MT trich tags or any USDA approved form of official identification. Please remember, MT trich tags are official identification tags and you must maintain records of when and where they were used. The Trich certification form (SV-69A) may be used as your record of tag application. MT trich tags are color coded based upon the year of the test. Tag color rotation is as follows:

<u>Trich Year</u>	<u>Test Period</u>	<u>Tag Color</u>
2023	Sept 1, 2023-August 31, 2024	Yellow
2024	Sept 1, 2024-August 31, 2025	Green
2025	Sept 1, 2025-August 31, 2026	White
2026	Sept 1, 2026-August 31, 2027	Orange
2027	Sept 1, 2027-August 31, 2028	Blue

In test year 2028, the tags will once again be Yellow, and the sequence will repeat.

If you are collecting samples to be held pending the results of a bull sale to determine which animals require trich test If any, MT trich tags should not be used as official identification.

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Sampling:

Sample procedures must be done with exacting care. Inoculation and transport of samples must be appropriate to be confident of test results. Everything used in the sampling process must be clean and sterile and single use to prevent the possibility of organism spread between animals. A blood tinged sample is not required.

The following materials should be assembled in advance of each collection:

- Sterile AI pipettes that are 26-30 inches long for each animal
- Suction device – sterile syringe or sterile rubber bulb for each animal
- Single use gloves
- Sterile saline without antimicrobial agents (for WET collection only)
- Transport media – The *TF InPouch* can be used for both culture and PCR testing. The *TF-Transit* tube is only appropriate for PCR testing.
- Clean scissors to trim excess preputial hair
- Care must be taken to avoid contamination of equipment – keep a bucket of disinfectant close by to clean hands and equipment.
- Paper towels/ towels to wipe excess debris from sheath

Overview of Basic Procedures:

Sample Collection:

1. Clean the underside of the animal, removing contamination around the preputial orifice. Trim sheath hairs and disinfect the scissors.
2. The sample is smegma from the glans penis obtained by performing a vigorous back and forth motion scraping of both the preputial and glans penis mucosa. A vigorous but not violent scraping of at least 30 strokes is recommended.
3. A satisfactory sample may contain a small amount of blood.
4. Insert the pipette into the preputial opening and advance to the fornix.
5. Use one of two procedures – Wet or Dry (Best results have been obtained collecting the mucus sample using no saline).

Wet:

- a. Use 3-5 ml sterile saline (without antimicrobial agents) drawn into a sterile syringe affixed to a sterile pipette. The solution is instilled into the prepuce after advancing the pipette to the fornix, just prior to scraping the mucosal surface.
- b. During the process, continued suction is applied using the syringe to retrieve as much of the instilled saline as possible.
- c. Remove the pipette and expel 1 ml of the sample into the top of the *TF InPouch* or the *TF-Transit* tube.
- d. If necessary, incorporate the contents of the upper chamber into the lower as per the *TF InPouch* directions and fold the wire strips to seal the chamber.

Dry:

- a. Collect the smegma into a sterile pipette, keeping the plastic sheath in place and using a sterile syringe or rubber bulb; apply negative pressure to aspirate the sample into the pipette.
- b. The sample is washed out of the pipette using the solution in the top of the *TF InPouch* or the *TF-Transit* tube.

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- c. If necessary, incorporate the contents of the upper chamber into the lower as per the *TF InPouch* directions and fold the wire strips to seal the chamber.

Inoculating the transport media is an important step:

- Only 1 sample should be inoculated into a pouch or tube. Pooling, if requested will be completed at the diagnostic laboratory.
- The expiration date of the media must remain valid during the incubation period. Expired pouches are not guaranteed by the manufacturer.
- All samples must be submitted in either a TF transport tube or a TF In-pouch. Saline or other media are not acceptable.

Keep the samples at room temperature (65-80°F) during the collection and shipment process. Protect the samples from direct sunlight and temperature extremes.

Prepare the sample for shipment:

- Ship samples the day of collection when possible.
- Samples should be maintained at room temperature during shipment.
- Do not ship samples with cold packs. DO NOT FREEZE SAMPLES.
- Ship samples by the fastest means possible, samples must reach the lab within **72** hours from the time of collection.
- If possible, avoid collecting samples near the weekend or before a holiday. If this cannot be avoided, samples can be incubated over the weekend or sent on during non-freezing temperature seasons.

Acquiring Test Results:

TF InPouch culture tests for the Montana Trich program must be sent to an AAVLD accredited laboratory for consistency in incubation and reading. Any state vet AAVLD lab is accredited. If *TF InPouch* cultures are positive for Trich species they should be sent on immediately to a lab that performs PCR for a definitive diagnosis. Submit samples with a complete and legible SV-69A submission form.

Preferred labs for PCR at this time are ***MT DOL Lab, WY State Lab in Laramie, WY and CSU in Rocky Ford, CO*** due to quality control and consistency. Samples for PCR can be submitted in either a *TF InPouch* or a *TF-Transit* tube. Samples should be shipped to the lab the same day as the collection occurred. UPS and Fed/Ex are best but mail is okay. Lab turnaround is 5 to 7 business days once received. Always check prices in advance, as they are subject to change.

MT DOL Veterinary Diagnostic Laboratory:

P.O. Box 997
Bozeman, MT 59771-0997
406-994-4885

UPS/FEDEX Address
South 19th and Lincoln
Bozeman, MT 59718

CSU Diagnostic Lab:

2747 Rd 21
Rocky Ford, CO 81067
719-254-6382

Wyoming State Veterinary Laboratory:

1174 Snowy Range Road
Laramie, WY 82070
307-766-9925

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Or if reading in the Clinic:

TF InPouch tests can be read by *experienced* culture reading veterinarians. **Incubate at 35°-36° C**

- *To proceed directly to 10x microscopic examination for immediate specimen reading, stand the pouch vertically for at least 15 minutes after collection and read the bottom chamber.*
- Before reading the pouch it is suggested to “pull the pouch up and down across the edge of a table approximately 3-4 times” according to InPouch directions. Keep the packet upright at all times once the lower chamber is inoculated unless reading the sample.
- Proceed with the proper timing of 24-hour interval examinations for 6 days. BIOMED says that 98% of pouches will be positive at 5 days and 2% at 6 days *if T. foetus* is present.

REMEMBER:

- Single use equipment
- Appropriate technique with at least 30 collection strokes, slight amount of pink is okay.
- Proper and timely transport in an appropriate media
- Complete and legible paperwork
- Official individual animal identification is required
- **Trich is reportable within 24 hours of a positive diagnosis**

Both the *TF InPouch* and the *TF-Transit* tube are available from:

BioMed Diagnostics
1388 Antelope Road
White City, OR 97503
Phone: (541) 830-3000
Toll Free: (800) 964-6466
Fax: (541) 830-3001

<http://www.biomeddiagnostics.com/pilot.asp?pg=Tfoetus> main page

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