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# STATE OF MONTANA

STEVE BULLOCK, GOVERNOR

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## ***Veterinarian Protocol for Trichomoniasis Testing in Bulls September, 2014***

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 and expiration dates.*

### **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.\**
- Animals 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>
2013	Sept 1, 2012-August 31, 2013	Orange
2014	Sept 1, 2013-August 31, 2014	Blue
2015	Sept 1, 2014-August 31, 2015	Yellow
2016	Sept 1, 2015-August 31, 2016	Green
2017	Sept 1, 2016-August 31, 2017	White

In test year 2018, the tags will once again be Orange, 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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## **Materials Needed:**

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:

- Clean, unused AI pipettes that are 26-30 inches long for each animal
- Suction device – clean, unused syringe or 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

## **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 c* or the *TF-Transit* tube.
- 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.

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Inoculating the transport media is an important step:

- Only 1 sample should be inoculated into each *TF-Transit* tube or *TF InPouch*. Pooling of samples, if requested will be completed at the diagnostic laboratory.
- The expiration date of the media must remain valid during the incubation period. Recently expired pouches (<1 week) may be accepted as normal samples at the diagnostic lab's discretion. Please contact the lab PRIOR to submission.
- Expired pouches (>1 week, but not more than 1 month expired), lactated ringers, and PBS may be used as transport media ONLY. Please note, this can only be done for PCR testing. Samples submitted in any of these media MUST arrive at MVDL WITHIN 24 HOURS of collection where they will be re-inoculated into appropriate media for incubation. Practitioners will be charged for the new media.

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 to the MDOL Veterinary Diagnostic Laboratory:**

- 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 in current media must reach the lab within **72** hours from the time of collection. Samples in "transport media" must reach the lab within 24 hours of 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, when inoculated into currently dated media.

**Acquiring Test Results:**

*Both culture and PCR tests* for the Montana Trich program must be sent to an AAVLD accredited laboratory for consistency. Samples for culture must be submitted in *TF InPouches*. 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.

MDOL Veterinary Diagnostic Laboratory:

P.O. Box 997

Bozeman, MT 59771-0997

406-994-4885

UPS/FEDEX Address

1911 West Lincoln

Bozeman, MT 59718

If you are using a lab other than the MT DOL VDL, please remember:

- Each lab may have different recommendations on submission protocol. Please be sure to contact the lab in advance to learn their policies.
- Samples positive by culture, must be confirmed by PCR.
- Trich is a reportable disease in Montana. Positive tests must be reported to MDOL within 24 hours.

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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)

[http://www.biomeddiagnostics.com/pilot.asp?pg=TF\\_studies2](http://www.biomeddiagnostics.com/pilot.asp?pg=TF_studies2) (video)