

New Test Announcement:
Direct real-time PCR, *Tritrichomonas foetus* RNA
 Division of Animal Industry



Dear Clients:

Effective February 5, 2024, the Nevada Department of Agriculture’s (NDA) Animal Disease Laboratory (ADL) will begin offering testing for *Tritrichomonas foetus* (*T. foetus*) by direct real-time PCR (RT-rtPCR) using Phosphate-buffered saline (PBS) or saline collections. This new assay has shown increased sensitivity of *T. foetus* detection in smegma samples by targeting the 5.8S ribosomal RNA of the *T. foetus* organism. Samples collected in PBS or saline will no longer require incubation. These changes will improve sensitivity, reduce turnaround time (TAT), and reduce costs associated with collection devices.

The ADL will continue to offer testing on Biomed InPouch® TF culture pouches and tubes utilizing the current assay until December 1, 2024. This option will only be available in our Sparks ADL. Consult the NDA ADL Test Menu available at agri.nv.gov/animal for detailed collection criteria.

Discipline	Molecular
Test Description	This direct real-time PCR (RT-rtPCR) detects <i>Tritrichomonas foetus</i> RNA – Bovine.
Test Schedule	Monday – Friday, TAT 3-4 business days.
Specimen	Smegma collected in 2 – 3mL of sterile phosphate-buffered saline (PBS, 1X) or 0.90% sterile saline in a sterile 15ml plastic conical tube.
Special Instructions	<ul style="list-style-type: none"> ▪ PBS and saline may not be used for Trichomonas Culture. ▪ Do not incubate samples. Ship samples as soon as possible after collection. ▪ Samples may be stored and shipped refrigerated. Refrigerated samples must arrive in the laboratory within 5 days of collection. Acceptable temperature range: 35°F-65°F. ▪ Samples may be stored and shipped frozen. Frozen samples must arrive in the laboratory within 7 days of collection and be received at a temperature less than 42 °F. ▪ Pooling will only be performed by laboratory personnel. Three to five individual samples from the same premises may be pooled for testing. ▪ If a pool tests positive, all samples will be retested individually and charged the individual <i>T. foetus</i> PCR price of \$25.00 each. ▪ Contact the laboratory for submissions of 100 samples or more. ▪ Clients should schedule shipments to avoid weekend and holiday delivery of samples to the laboratory.
Reasons for rejection	<ul style="list-style-type: none"> ▪ Unlabeled samples. ▪ Samples that leak in transit. Ensure that all caps are properly affixed to tubes. ▪ Samples received out of the shipping temperature guidance may not be considered official. If testing is requested, the results will indicate “Unofficial.”
Cost	\$25.00 individual sample or \$40.00 for sample pools of 3-5
Submission Forms	If submitting using older forms, clearly indicate on the form <i>Pooled PCR Testing</i> . Contact the ADL laboratory at (775) 353-3709 for new electronic forms. Check our website at agri.nv.gov for future updates.

Frequently Asked Questions

What collection devices are allowed for testing?

1. Direct smegma collected in PBS, 1X (Preferred)
2. Direct smegma collected in .90% sterile saline
3. The ADL will continue testing Biomed's InPouch® TF Bovine or Tube **until December 1, 2024**. This option will only be offered at our Sparks location. Please note this test is a different methodology than the direct rtPCR for PBS and saline.

How do I ship samples to the ADL for a VALID official test?

PBS or Saline (tested by Direct rtPCR RNA):

- **Method 1 Refrigerated:** Do not incubate. Ship refrigerated within 5 days of collection. The samples must be maintained at 35-65°F.
- **Method 2 Frozen:** Do not incubate. Ship frozen within 14 days of collection. The samples must be maintained at ≤ 42 °F. Ensure that all frozen tubes have direct contact with frozen icepacks.

Biomed InPouch® TF Bovine Pouch or Tube (tested by rtPCR DNA):

- **Method 1:** Ship the individual samples by overnight courier to arrive at the lab within 48 hours of collection. For this method ship samples at room temperature (no ice pack). Samples can also be directly delivered to the laboratory. Samples must be maintained at 65-90°F. If real-time PCR is requested, the ADL will incubate, freeze, and pool samples.
- **Method 2:** Incubate the samples at 37 degrees centigrade for 24 hours, then freeze the samples. For this method, ship with an ice pack(s) at your convenience using an overnight courier. Samples must arrive at ≤ 42 °F.

All PBS or saline samples may be shipped to or dropped off at one of the following locations:

Nevada Department of Agriculture
Animal Disease Laboratory
405 S. 21st Street
Sparks, NV 89431

Nevada Department of Agriculture
Animal Disease Laboratory
4780 East Idaho Street
Elko, NV 89801

Are there any restrictions for pooled testing?

1. ADL will not pool multiple owners in a single pool.
2. Herds with a history of infection within the past 12 months should not be pooled.
3. Herds that are currently under a quarantine or hold order for trichomoniasis, and/or part of an epidemiological investigation in which individual PCR is required will not be pooled.
4. Always check the state of destination's entry permit requirements before ordering pooled testing. Some states will not accept pooled results.

Guidance on ordering supplies

Supplies may be ordered through MWI or comparable products are available through Fisher Scientific. For sites ordering tubes, the laboratory recommends RNase and DNase-free tubes. If PBS 10x is purchased, it should be diluted to 1X. Contact the ADL for support or questions at 775-353-3709.

MWI Animal Health – Amerisource Bergen	Catalog	Est Cost	~Price Each
Premium Centrifuge Tube, Sterile 15mL RNase and DNase-free, 50e	101306	\$13.90	\$ 0.28
Phosphate Buffered Saline (PBS), 1X, Cytiva Hyclone™, 500mL	051213	\$38.96	\$ 0.16
Sterile Saline Solution 0.9%, 500mL	510223	\$4.07	\$ 0.02