

BADDL offers real-time PCR assay for the detection of *Tritrichomonas foetus*: Sample type

It is important to collect the appropriate samples for *Tritrichomonas foetus* qPCR. The preferred **bovine** specimens for the *Tritrichomonas foetus* qPCR assay are a fresh preputial swab/wash, smegma, or cervical-vaginal mucus inoculated into an InPouch™ or qPCR, or smegma in sterile PBS (phosphate buffered saline) for direct qPCR.

The preferred **feline** specimens for the *Tritrichomonas foetus* qPCR assay are rectal swabs or fresh feces inoculated into an InPouch™ TF Feline.

Up to five samples may be pooled to save costs. However, InPouches must contain *individual* samples; pooling **MUST** be conducted by BADDL staff. **Samples for direct smegma PCR cannot be pooled for testing.** Culture is only available for EXPORT cases when the country (or state) of destination will not accept PCR test results.

Bovine smegma collection procedure

Begin with a labeled, unexpired InPouch TF and a sampling device for each animal to be sampled. Safely restrain the bull. Clean debris from the preputial orifice and trim the sheath hair. Lavage the sheath with a minimal amount of saline to clean away dirt and manure to prevent contamination.



With a new 12cc syringe and a guarded pipette, introduce the pipette to the sheath to about the distal third of the preputial cavity. Advance the collecting pipette through and beyond the protecting tube to the preputial fornix.

Collect the sample by scraping the pipette back and forth in short strokes on the mucosa of the distal penis and fornix area while applying suction with the syringe to move smegma into the pipette. Aggressively apply a steady vacuum, but not so aggressively as to draw blood. Fifteen to thirty strokes of the pipette are required to obtain adequate sample.

Retract the pipette back into the protecting tube and remove the device. Insert the pipette with the sample into either:

- **InPouch sample:** Open the upper opening of the InPouch and eject the sample. Pipette some fluid back into and out of the pipette to ensure that the sample is fully ejected. Close the pouch by rolling down from the top, making sure that all the liquid is squeezed into the lower portion of the pouch. Secure the pouch with the tabs.

- **Direct PCR sample:** Pipette the smegma sample into a 4 mL test tube (red top tube or similar tube) with 1.5 mL of sterile PBS (Phosphate Buffered Saline). The collection pipette should be rinsed with the PBS by aspirating several times to ensure all of the smegma sample is deposited in the tube. The tube should be labeled as direct smegma with bull ID/ date collected.

Feline sample collection

Swab rectally with a sterile swab and express the sample in a labeled, unexpired InPouch TF Feline.

Alternatively, add approximately 0.03 grams of fresh feces to a labeled, unexpired InPouch TF Feline.



Close the pouch by rolling down from the top, making sure that all the liquid is squeezed into the lower portion of the pouch. Secure the pouch with the tabs.

Shipping/Transport

Include a BADDL submission form in a zippered plastic bag inside the container or between the container and the box. Be sure to write "*Trich. foetus* PCR" under "Tests Requested". Date and time of collection must also be included on the form.

In Pouch Samples

- Wrap each InPouch in a paper towel and seal inside a zippered plastic bag. Place this in a shipping container with packing materials such as bubble wrap to prevent excessive shifting during transport.



- **InPouch samples MUST be:**
Received at the lab within 48 hours of collection, protected from light, and shipped in the package with a temperature range of 15-37°C (50-100°F).

- If samples cannot be transferred to the lab within 48 hours they may be incubated at 36-37°C (96.8-98.6°F) for 48 hours, at which point they can be kept refrigerated for transfer to the lab. If the samples have been incubated, indicate on the submission form the number of hours incubated and the temperature.
- DO NOT refrigerate samples unless they have been incubated for at least 48 hours
- If the samples have completed the 48-hour incubation, they should be shipped overnight on ice packs. BADDL prefers to perform the *Tritrichomonas* sample incubation at the lab where conditions are tightly controlled and monitored for best test performance. Therefore, reasonable effort should be made to send samples to the lab within 48 hours of collection.

Smegma direct PCR sample MUST be:
Received at the lab within 48 hours of collection and shipped chilled overnight. Shipping address: BADDL – 2700 N. John Young Parkway, Kissimmee, FL 34741

Turnaround Time

Two - 3 business days for direct PCR. *Trich* InPouch samples must be incubated for 48 hours prior to testing by qPCR. Test

results can be expected within 2-3 business days following the incubation period. Therefore, total turnaround time is approximately 2-5 days. Please contact the Molecular Diagnostics Section at BADDL (321) 697-1400 with any questions.

Cost

Individual Trich RT-PCR tests are \$17 each. Each pooled test is \$30. **Samples for direct smegma qPCR cannot be pooled for testing.**

- If a pooled sample returns positive results, each sample from the pool will be retested individually at \$17 each. Samples will not be retested, and clients will not be charged without client permission.
- If the sample number is ≤ 2 , the lab will run as an individual PCR.
- If the sample number is between 3-5, the lab will run a pooled test if individual request is not indicated on the submission form. If the pooled test is positive, individual tests will be performed to identify the positive samples, and the client will be charged for both pooled and individual tests.

Tritrichomonas culture is only available for EXPORT cases. If the culture test is positive, PCR confirmation for *T. foetus* will be performed at a cost of \$27 (\$17 PCR + \$10 culture).



ADDRESS AND CONTACT

Bronson Animal Disease Diagnostic Laboratory

Mailing Address
2700 N. John Young Parkway
Kissimmee, FL 34741
Phone: (321) 697-1400
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www.FreshFromFlorida.com/BADDL

Tritrichomonas foetus (Trich) Testing Real Time Polymerase Chain Reaction (RT-PCR)



Florida Department of
Agriculture and Consumer Services