

ISDA Animal Health Laboratories
--

SOP Title: <i>Trichomonas foetus</i> Identification
--

Contributor: Daniel Salmi and Marilyn Simunich

Document #: BAC3

Department: Bacteriology

DIAGNOSIS OF TRICHOMONIASIS:

Diagnosis of trichomoniasis is made when *Trichomonas* organisms are observed in the smegma or preputial flush samples of bulls, or the uterine or vaginal fluid of cows. The organisms may be observed by direct microscopic examination of the fresh samples, by examination of culture media inoculated with infected material, or by the detection of *T. foetus* DNA through Polymerase Chain Reaction (PCR).

SAMPLE COLLECTION:

Note: Only veterinarians registered with ISDA, Division of Animal Industries shall collect samples for official tests per Idaho Administrative Procedures Act (IDAPA) 02.04.29 Rules Governing *Trichomonas*.

1. The preferred sample from the male is smegma from the glans penis. This can be obtained by performing a vigorous back and forth scraping motion along the glans using a sterile insemination pipette while applying negative pressure with an attached 20 or 30 mL syringe. This material is then inoculated into a medium suitable for transport to the laboratory.
2. The preferred sample from the female is the cervical mucus or uterine secretions. These samples can easily 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. This material is then inoculated into a medium suitable for transport to the laboratory.

In-Pouch medium for *T. foetus*:

The Biomed InPouch TF culture system is the only officially recognized media for the culturing of bovine *Tri-trichomonas foetus* organisms in the state of Idaho.

The InPouch may be utilized for PCR testing.

At least 5 mm of sample should be collected at the bottom of the pouch. On the first read, any pouches that appear like they have less than 5 mm of sample present should be measured and marked. If less than 5 mm, than the sample will be rejected. If 5 mm or greater, than the sample is OK.

TF Transit Tube for PCR:

A Biomed TF transit tube may be inoculated with 0.5 – 1.0 mL of smegma scraping to be examined by PCR only.

OVERVIEW OF TESTING METHODS:

1. **Culture:** Collected samples shall be introduced into the InPouch TF and incubated at 37° C (98.6° F). Samples should be microscopically observed every other day for a period of 7 days. Culture testing can be done at ISDA - Animal Health Laboratory or at ISDA certified facilities by ISDA certified readers.
2. **qPCR:** Also known as quantitative real-time PCR, this method is believed to have several advantages over traditional culture testing; a) qPCR is generally considered to have greater sensitivity and specificity. b) qPCR results are typically generated within 2-3 days. c) qPCR can distinguish between *Tritrichomonas foetus* and other trichomonads. d) In some instances, one qPCR test may be accepted versus three separate culture tests. e) Inoculated pouches for qPCR are shipped cold (frozen), so there is no need for hand-warmers to maintain adequate organism growth/survival temperatures.

The collected sample is introduced into the InPouch TF system or the *T. foetus* transit tube. Samples submitted in other media, such as Diamonds (MDM), can contain PCR inhibitors and will be rejected. Inoculated media should be incubated at 37°C (98.6° F) for 18-24 hours. Samples should then be frozen and shipped on ice to the ISDA – Animal Health Laboratory or another accredited laboratory.

MEDIA INOCULATION:

InPouch TF: Inoculate the sample into the small upper chamber of the pouch and fold the wire strips down at least two folds to seal the upper chamber. Remember that at least 5 mm of sample should be collected at the bottom of the pouch.

1. If a direct microscopic examination is to be made of the upper chamber at the time of collection, position the chamber into the viewing apparatus and close the frame over the raised platform and the pouch. Observe microscopically using low power (10X objective) for the presence of motile trichomonads. Use high power (40X objective), if necessary, for confirmation. (If no direct microscopic examination is to be performed at this time, proceed directly to step 2).
2. Incorporate the contents of the upper chamber into the lower in the following manner: Squeeze or roll up the pouch from the bottom until the culture medium in the lower chamber ruptures the frangible (breakable) seal between the two chambers and washes into the upper chamber. Manipulate the liquid around slightly to mix the inoculum and medium, and then squeeze or “squeegee” the liquid back into the lower chamber. Express any air bubbles out of the lower chamber. This action helps to maintain the anaerobic quality of the medium. Roll the wire strips the rest of the way down (like a whirl-pak) to seal the lower chamber. Make sure you roll the wire strips up to the lower chamber.

TF Transit Tube: Inject the 0.5 – 1.0 mL smegma sample into the TF transit tube and replace cap securely.

SHIPPING AND HANDLING:**1. Shipping for Culture Testing:**

NOTE: The shipping and handling of the inoculated medium sample for culture is one of the most critical steps in trichomoniasis diagnosis. It is important to arrange shipping so that the samples arrive at the laboratory that will perform the testing within 48 hours of collection. Only those samples which are received at a certified diagnostic facility within 48 hours from time of collection will be considered for a valid culture test. Samples received after 48 hours from the time of collection will not be tested. Also note the Trichomoniasis Test and Report Form must have the “Date of Collection” completed or the sample(s) will not be tested.

- a. It is important to insure that inoculated media are not overly shaken or agitated during handling or transport back to the clinic or laboratory.
- b. The inoculated media should be kept as close as possible to general room temperature (65°F – 75 °F) until it is incubated. It is especially important to avoid overheating or freezing.
- c. All samples for culture sent to the ISDA – AHL will have their temperature measured upon arrival by infrared thermometer. If the samples are below 60°F or above 120°F, the veterinarian will be contacted and given the option of resubmitting the samples or having the ISDA – AHL perform qPCR on the samples.
- d. If the samples are to be shipped to another laboratory or clinic for examination, ship the inoculated pouches in insulated containers (no ice) that will protect the samples from extreme temperatures. Trichomonads are very susceptible to either freezing or overheating. Chemical hand warmers or micro-waved gel packs should be placed in the shipping containers if the ambient (outdoor) temperature is below 60°F.

2. Shipping for real-time PCR (qPCR) Testing:

- a. All samples submitted to ISDA - AHL for qPCR testing should be collected into the InPouch TF system or the TF transit tube.
- b. Before shipping, inoculated media should be incubated at 37°C (98.6°F) for 18-24 hours.
- c. The InPouch or TF transit tube media should then be frozen and shipped with gel “icepacks” to ISDA – AHL.

Note: If a veterinarian reads their own pouches and find a potential *T. foetus* positive pouch, the ISDA – AHL can perform a *T. foetus* confirmation qPCR on the sample. The pouch should be frozen, packed with gel “icepacks”, and shipped or sent by courier to the ISDA – AHL to arrive within 24 hours.

CULTURING PROCEDURES:

InPouch TF Pouch: Specimens arriving in the InPouch TF pouch by mail are microscopically examined upon arrival (See Exam Procedures below) and then put into the incubator. Specimens that were collected that day and brought in are put directly into the incubator. The pouches are incubated vertically (upright) at 37°C and examined until positive growth and confirmation occurs or until they have remained negative for 7 days (See Examination Procedures below). Specimens remaining negative for 7 days are reported as negative.

EXAMINATION PROCEDURES:

Note: All Trichomoniasis culture tests are considered official tests. **There are no “unofficial tests”**. Only laboratories approved by ISDA Division of Animal Industries shall test official Trichomoniasis samples per IDAPA 02.04.29. Lab personnel must be trained and certified by ISDA AHL personnel following the most current revision of SOP BAC5 Certification of Individuals for Trichomoniasis Microscopy Testing to examine samples for *Tri-trichomonas foetus* per IDAPA 02.04.29. Newly-trained personnel must pass a ‘one-time’ proficiency test administered 1 year from the initial training.

A clinic must also have an initial, one time only, on-site inspection to read Trichomonas samples. This inspection can be completed by ISDA field staff following the most current revision of SOP BAC6 Certification of Facilities for Trichomoniasis Microscopy Testing.

InPouch TF Pouch: For microscopic examination, place the bottom of the lower chamber of the pouch on the raised platform of the open viewing frame. Close and lock the frame over the pouch. Trichomonads generally will first be found slightly above the bottom border of the chamber. (NOTE: If samples are opaque, mix and try to read in the clear spaces.) The pouch and frame are then placed on the microscope under a 10X objective (100 power) and examined for typical motile organisms (see Interpretation of Results below) in the following manner:

1. The day samples are received is Day 0 (zero). If samples have been in shipment for more than a few hours, it is suggested to examine the samples the day they arrive. Incubate the samples vertically (upright) at 37°C (98-99°F). Samples should be closely examined on days 1, 3, 5 and 7. The results for that day’s reading are recorded on the Official “Trichomonas Test and Report Form” (SOP ADMFRM8) and the “Trichomonas Test and Report Form Continuation Sheet (SOP ADMFRM9) if needed. Record the date read at the top of the column above the column number. Then record the results for each sample in the column for that day’s reading. The final results are recorded at the end of day 7, or earlier for those samples on the test form that have already turned positive. **(Note: If there are a mixture of positives and negatives found on the same day, call the owner and report the positives prior to day 7.)**
2. Upon completion of the 7 days of testing, the laboratory performing the test fills out the summary information and the “certified reader” signs the forms. The forms are then forwarded according to the distribution labels at the bottom of the form.

3. Occasionally the trichomonads may progress through a rapid growth (log) phase and die off very quickly, it is suggested (but not required) that the samples also be given a brief look on days 2, 4 and 6 to check for motility.

INTERPRETATION OF CULTURE RESULTS:

Positive: A sample is considered positive when viable, motile trichomonad organisms are observed either upon direct microscopic examination of the sample when collected, or in the culture medium on any of the reading days. Samples may be sent for *T. foetus* PCR confirmation. Trichomoniasis is a reportable disease in the state of Idaho and it is the responsibility of the individual to report a positive sample to the state veterinarian.

Negative: A sample is considered negative when **no** viable, motile trichomonads are observed in the culture medium during **any** of the reading days and after 7 days incubation has been completed. **(Note: If no viable trichomonads are seen upon direct microscopic exam when the sample is collected, that is common. A sample must be cultured for the full 7 days before it can be called negative.)**

All pouches should be retained for 7 days after the final read. This would allow samples to be inspected if questions arise.

Re-Test: When the *Trichomonas* organism dies, it immediately loses its motility. Its morphology, however, will degenerate more slowly. With a rapidly growing or heavily inoculated sample, the trichomonad organisms can sometimes overgrow the medium and die off within a 36-48 hour time period. That is the reason for the every-other-day reading schedule; you should catch the organisms sometime during their growth phase. However, if you encounter a culture sample with abundant non-motile organisms of typical trichomonad size, mark the test chart as “RE-TEST” for that sample and request that a second specimen be submitted for that animal as soon as possible.

[Reasoning: Some yeasts and spores will be of similar size and morphology as a dead (non-motile) trichomonad. Thus rather than incorrectly calling the animal “Positive”, a second sample should be immediately requested and read every day (in case it’s a rapidly growing trichomonad) to observe if actual, motile trichomonad organisms are present. If no viable, motile trichomonad organisms are found upon the re-culture, the animal is negative.]

INTERPRETATION OF qPCR RESULTS are in the most current revision of SOP MOL9 Real-Time PCR Testing for *Trichomonas foetus* on file at ISDA – AHL.

SAMPLE DISPOSAL:

Pouches may be discarded at the end of the 7-day retention period and **all** should be inactivated (regardless of whether the final results were positive or negative) in accordance with the EPA and OSHA requirements for disposal of biological wastes. This is best accomplished by autoclaving the pouches prior to discarding. If an autoclave is not available or if autoclaving is not practical, inactivate by adding Clorox, Nolvasan or some other disinfectant to the pouches and shaking vigorously prior to disposal.