ISDA Animal Health Laboratories

SOP Title: *Tritrichomonas foetus* Identification
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Note: The title of this document is officially "Protocol for Trichomonas Diagnosis in Cattle". It is incorporated by reference by IDAPA 02.04.29-Rules Governing Trichomoniasis.

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 examination of culture media inoculated with infected material, or by the detection of *T. foetus* DNA through Polymerase Chain Reaction (PCR).

OVERVIEW OF TESTING METHODS:

1. **Culture**: Collected samples shall be introduced into the InPouch™ TF (InPouch) and incubated at 37º C (98.6º 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. **~~q~~PCR**: ~~Also known as quantitative real-time PCR,~~ T~~t~~his method is believed to have several advantages over traditional culture testing; a) ~~q~~PCR is generally considered to have greater sensitivity and specificity. b) ~~q~~PCR results are typically generated within 2-3 days. c) ~~q~~PCR can distinguish between *Tritrichomonas foetus* and other trichomonads. d) In some instances, one ~~q~~PCR test may be accepted versus three separate culture tests. e) Inoculated and incubated InPouches or TF Transit Tubes (Transit tube) for ~~q~~PCR 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 system~~-~~, ~~or the~~TF Transit tube, or other ISDA approved transport system. Samples submitted in other media, such as Diamonds (MDM), can contain PCR inhibitors and will be rejected. Inoculated InPouches or Transit tubes should be incubated at 37°C (98.6º F) for 18-24 hours. All samples should be frozen and shipped on ice to the ISDA – Animal Health Laboratory or another accredited laboratory.

   The ISDA Animal Health Lab does offer pooled Trichomonas foetus PCR testing. Submit Transit tubes (preferred) or InPouches as you would for individual testing. DO NOT POOL SAMPLES PRIOR TO SUBMISSION! The ISDA Animal Health Lab DOES THE POOLING! We will pool a maximum of 5 samples. If the pool tests positive, the samples are then tested individually at the individual PCR test price.
   The ISDA Animal Health Lab will only use United States Department of Agriculture (USDA) approved *Tritrichomonas foetus* PCR test kits.

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OFFICIAL MEDIA:

1. **InPouch TF medium for culture:**
   a. 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.
   b. The InPouch may be utilized for PCR testing.
   c. 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, the sample will be rejected. If 5 mm or greater, the sample is OK.

2. **TF Transit Tube for PCR:**
   a. A Biomed TF transit tube may be inoculated with 0.5 – 1.0 mL of smegma scraping to be examined by PCR only.

3. **Other ISDA approved transport systems for *T.foetus* PCR testing:**
   a. **Other validated products that have been approved by the ISDA State Veterinarian can also be used to submit samples for PCR testing.**

SAMPLE COLLECTION:

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 an ISDA approved media and then ~~InPouch or Transit tube for~~ transported 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 an ISDA approved system and then ~~InPouch or Transit tube for~~ transported to the laboratory.

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 Trichomoniasis.

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MEDIA INOCULATION:

InPouch:

1. Prepare InPouch
    a. Manually express the liquid so that all the liquid is in the lower chamber. Open the pouch by tearing off the top. There is a pre-formed score to facilitate tearing. Use the integral white tabs to open and secure the mouth of the pouch open.
2. Inoculate Sample
    a. Insert the sample into the upper chamber of the pouch (0.5 – 1.0 mL of specimen). Squeeze a small amount of liquid from the lower to the upper pouch chamber to flush the sample. Minimize the introduction of bubbles or foam.
3. Integrate Sample
    a. Express the entire contents of the InPouch into the lower chamber. Avoid trapping air. Roll the pouch top tightly, until the wire-tape is at the top of the label. Fold the wire tape ends tabs to seal the pouch.

Transit Tube:

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

Other ISDA approved transport systems:

1. Follow manufactures recommended protocol.

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SHIPPING AND HANDLING:

1. **Shipping for Culture Testing:**
    a. The inoculated media should be kept as close as possible to general room temperature (65ºF – 75 ºF) until it is incubated. It is especially important to avoid overheating or freezing.
    b. All samples for culture sent to the ISDA – AHL will have their temperature measured upon arrival by infrared thermometer. If the samples are below 60ºF or above 120ºF, the veterinarian will be contacted and given the option of resubmitting the samples or having the ISDA – AHL perform ~~q~~PCR on the samples.
    c. 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~~warmed gel packs should be placed in the shipping containers if the ambient (outdoor) temperature is below 60ºF.

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.

2. **Shipping for ~~real-time~~ PCR ~~(qPCR)~~ Testing:**
   a. All samples submitted to ISDA - AHL for ~~q~~PCR testing should be collected into the InPouch system, ~~or a~~ TF Transit tube, or other ISDA approved transport system*.

   b. Before shipping, inoculated InPouches or Transit tubes should be incubated at 37°C (98.6ºF) for 18-24 hours.

   c. The InPouch or Transit tube media should then be frozen and shipped with gel "icepacks" to ISDA – AHL.

   *Follow manufacturers recommended protocol.

   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 ~~q~~PCR 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 REQUIREMENTS AND PROCEDURES:

1. All Trichomoniasis culture tests are considered official tests. **There are no "unofficial tests"**.
2. Only laboratories approved by ISDA Division of Animal Industries shall test official Trichomoniasis samples per IDAPA 02.04.29.
3. Lab personnel must be trained and certified by ISDA personnel following the most current revision of SOP "Certification of Individuals for Trichomoniasis Microscopy Testing to examine samples for *Tri-trichomonas foetus* per IDAPA 02.04.29".
4. Clinics/Veterinarians will then be placed on an every 3 year proficiency test schedule.
5. A clinics facilities 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 "Certification of Facilities for Trichomoniasis Microscopy Testing".

Incubation and Reading Schedule

1. After collection, pouches should be incubated vertically (upright) at 37ºC for 18-24 hours before the first read.
2. The pouches are examined every other day for a total of four reads or until positive growth is identified or until they have remained negative for 7 days.
3. Specimens remaining negative for 7 days are reported as negative.

Microscopic Examination Procedure

1. 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.)
2. The pouch and frame are then placed on the microscope under a low power (higher power if needed) and examined for typical motile organisms.
3. The results for that day's reading are recorded on the Official "Trichomonas Test and Report Form" and the "Trichomonas Test and Report Form Continuation Sheet if needed.
4. 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.
5. 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. Notify the submitter of the pouch or the owner of the bull in addition to the state veterinarian and or the AHL director.
    a. **Report positive results as soon as they are found, i.e. if any pouch of a multi pouch submission from one herd is found positive, it should be reported before the final day 7 read).**
6. 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.

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 at room temperature after the final read. This would allow samples to be tested by ~~q~~PCR 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. Therefore, 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 ~~q~~PCR RESULTS are in the most current revision of SOP MOL9 Real-Time PCR Testing for *Tritrichomonas foetus* on file at ISDA – AHL.

SAMPLE DISPOSAL:
Pouches may be discarded at the end of the 7-day retention period and the contents should be sterilized (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.