



## CALIFORNIA ANIMAL HEALTH AND FOOD SAFETY LABORATORY SYSTEM

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**The CAHFS laboratory in Davis is now accepting samples for *Tritrichomonas foetus* qPCR, also known as real-time PCR. Our expectation is that this will help to streamline testing. The details regarding submission of samples for this test can be found below.**

- 1) All samples must be a preputial scraping inoculated into InPouch media. Preputial washes submitted in saline are not acceptable for this testing as the test has not been validated with this specimen.
- 2) Inoculated InPouches can be sent to CAHFS for 24 hr incubation at CAHFS and subsequent qPCR. These samples should be sent at ambient temperatures between 65°F and 95°F and need to be received within 48 hr. after collection. Unfortunately, CAHFS cannot receive and store samples on weekends and holidays so it is best to ship samples by Thursday.
- 3) Inoculated InPouches can be incubated in the practitioner's office for 24 hr. The entire pouch should then be frozen and shipped to Davis CAHFS. The frozen pouch should be shipped on ice or with frozen gel packs and be received within 24 hr. The temperature of the sample must not exceed 55°F on arrival at the laboratory. Unfortunately, CAHFS cannot receive and store samples on weekends and holidays so it is best to ship samples by Thursday.
- 4) If samples arrive outside the acceptable time and temperature range, the samples will still be processed but a disclaimer will be added to the test result and it will not be considered a regulatory test unless the sample is positive for *T. foetus*.
- 5) The laboratory will still accept samples for routine culture, either preputial washes submitted in saline or inoculated InPouches. These samples should be received within 48 hr of collection. qPCR will not be performed on these routine cultures. However, if the culture becomes positive, *T. foetus* will be confirmed with our conventional PCR test at no charge in order to rule out fecal trichomonads. Alternatively qPCR can be requested but with a charge of \$25.
- 6) CDFA approved practitioners can continue to do routine cultures in their laboratories. Positive samples can still be sent to CAHFS for confirmatory PCR on cultures incubated in the practitioner's office as we do currently using conventional PCR at no charge. Alternatively qPCR can be requested for a charge of \$25. For confirmation, the entire pouch should be frozen and sent to the Davis laboratory as described in #3 above regardless of the test type requested.
- 7) CAHFS, Tulare and CAHFS, San Bernardino will continue to perform Diamonds media *T. foetus* cultures and InPouch cultures but neither lab is currently performing the conventional PCR or qPCR. They can forward samples to CAHFS, UC Davis for these tests.

### Frequently asked questions

What is qPCR/ real-time PCR?

*qPCR is a type of PCR that uses a different method to detect amplified trichomonad DNA. It is a more sensitive test than conventional PCR and is in use in a number of laboratories to test for T. foetus. It is sensitive enough that one single qPCR test is equivalent to 3 conventional cultures.*

Why should I consider using qPCR for *T. foetus* detection?

*Detection of T. foetus using qPCR has the capability of detecting an infected bull with a single test rather than the 3 weekly cultures. Consequently, testing is much faster.*

How much is qPCR testing?

*The cost for qPCR testing is \$25/sample, in the state of California.*

What if I have many samples to test?

*CAHFS can test these samples; however, if more than 30 samples are to be submitted for qPCR we request that the microbiology laboratory be contacted before shipping.*

How often is the qPCR test run at CAHFS, UC Davis?

*Testing will be performed routinely 1 to 2 times per week. If you need a sample tested more quickly, a “stat” charge will be added; it is an additional \$25/sample.*

What forms do I use to submit a sample for *T. foetus* qPCR testing?

*The CDFA Trichomonosis Reporting Form and a CAHFS Trichomonas InPouch Submission form are needed for testing. The latter will be available on our website.*

Why is the InPouch the only sample acceptable for qPCR?

*This is the only sample for which the qPCR has been validated.*

Why are there strict temperature restrictions on the InPouch transportation?

*Live trichomonads are quite sensitive to low and high temperatures. Also, once *T. foetus* dies, it contains DNAses that will rapidly degrade DNA leading to false negative results. Thus, it is important that the pouches, once frozen, remain frozen, or at least cold, so the DNA does not degrade.*

Can I still collect a preputial wash submitted in saline and have it cultured for *T. foetus* at CAHFS?

*Yes, CAHFS will still carry out culture of preputial washes using Diamond’s medium. However, this sample cannot be used for the qPCR test?*

Can I still culture for *T. foetus* in my clinic?

*Yes, conventional culturing can still be performed at your clinic. You have the option of following the culture for the usual 5 days or the sample can be frozen after 24 hr of incubation and sent to CAHFS. You must report the results from culturing samples in your clinic to CDFA; samples submitted to the CAHFS laboratory will automatically be reported to CDFA.*

What if I detect trichomonads in a culture at my clinic, can I still send it to CAHFS for confirmation as *T. foetus*?

*Yes, CAHFS will continue to perform the *T. foetus* confirmation testing using conventional PCR at no charge to the client. The pouch should be frozen and shipped on ice or frozen gel packs to the laboratory as described for #3 above.*

Can the qPCR test be used for export samples/bulls?

*Yes, some countries will accept this test; however, it is the responsibility of the submitting veterinarian to confirm that qPCR is an acceptable test for the particular country.*

What will the results be from the qPCR test?

*There are 4 possible results. Obviously there will be “positive” and “negative” results. An invalid result will be given if the internal control DNA fails to amplify indicating that there are inhibitors in the sample. An inconclusive result will be reported if there is very low amplification of DNA that means we cannot be certain *T. foetus* is present. This occurs when there is either very small amounts of *T. foetus* DNA in the sample or if there is low level of false amplification. Retesting of the bull is recommended for both the invalid and inconclusive result.*

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