Trichomonas Foetus DNA Test Kit

VetMAX™-Gold Trich Detection Kit

Catalog Number 4483869

Pub. No. 4485468 **Rev.** B

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product information

Name, intended use, and principle of the procedure

The Applied Biosystems™ Trichomonas Foetus DNA Test Kit (VetMAX™-Gold Trich Detection Kit, Cat. No. 4483869) is a highly sensitive, qualitative, real-time PCR assay for detection of *Tritrichomonas foetus* DNA isolated from enriched smegma samples.

Bovine trichomonosis is a sexually transmitted infection caused by *T. foetus* that results in significant monetary losses to the cattle industry in the United States and other parts of the world where open range management and natural breeding are practiced. *T. foetus* is a flagellated protozoan found in bovines that colonizes the vaginal, uterine, oviduct, and preputial epithelium, resulting in embryonic death, abortion, and infertility in the female. Although bulls are the main carriers of *T. foetus*, they remain asymptomatic for their entire lives. The VetMAX™-Gold Trich Detection Kit enables detection of venereal trichomonosis in carrier bulls.

The VetMAX™-Gold Trich Detection Kit can be used to test pools of up to 5 samples for the presence of *T. foetus*. Pool size should be determined by the testing laboratory, based on the prevalence of bovine trichomonosis in the area from which the samples were collected. Pooled samples yielding a positive result should then be tested individually to determine the infection status of each animal in the positive pool.

The assay is a single-well/tube, real-time PCR in which *T. foetus* DNA and Xeno™ DNA Control targets are amplified and detected in real time using fluorescent TaqMan® probes (hydrolysis probe chemistry). The kit includes:

- T. foetus-Xeno™ Control DNA Mix, to serve as a positive control for the real-time PCR components, and it is also used to set the cycle threshold (C_t) for evaluating test results.
- Xeno™ DNA Control, to serve as an internal control for the DNA isolation process, and it is also used to monitor for the presence of PCR inhibitors.
- T. foetus Primer Probe Mix, optimized for multiplex PCR amplification of both Xeno™ DNA Control and *T. foetus* targets.

Limitations

- Handle samples as recommended in Table 2 to prevent degradation of any *T. foetus* DNA that is present.
- Prepare pooled samples from no more than 5 individual samples.
- Pooling samples may cause loss of sensitivity of detection of infected animals with an individual C_t of ≥35.
- DNA extraction methods should yield DNA free of PCR inhibitors, which can prevent amplification of target DNA.
- Follow "Good laboratory practices for PCR and RT-PCR" on page 5 to prevent false positive amplifications due to contamination of test samples with PCR products.

Kit contents and storage conditions

Reagents for 100 25-µL real-time PCR tests are supplied.

Table 1 VetMAX™-Gold Trich Detection Kit

Component	Amount	Storage
2X qPCR Master Mix	1.375 mL	−30°C to −10°C
T. foetus Primer Probe Mix	110 µL	
Xeno™ DNA Control (10,000 copies/µL)	250 µL	
T. foetus-Xeno™ Control DNA Mix (1,000 copies/µL)	80 µL	
Nuclease-free Water	1.75 mL	−30°C to 25°C

Required materials not supplied

Item	Source[1]
Real-time PCR instrument and accessories, one of the following:	
7500 Fast Real-Time PCR System (96-well), running SDS Software v1.4 7500 Fast Precision Plate Holder, for 0.1 mL Tube Strips (Cat. No. A29252), or equivalent	Contact your local sales office.
QuantStudio™ 5 Real-Time PCR System, 96-well, 0.1-mL	Contact your local sales office.
Equipment	
Microcentrifuge	MLS
Laboratory mixer (vortex or equivalent)	MLS
Nuclease-free pipettors	MLS
2 ice buckets: • One for the PCR setup area where the master mix is prepared • One for the area where DNA may be present	MLS
Plates or tubes and caps	
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	4366932 (200 plates), 4346906 (20 plates), or equivalent
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL	4346907
MicroAmp™ Optical Adhesive Film	4311971 (100 covers), 4360954 (25 covers), or equivalent
MicroAmp™ Fast 8-Tube Strip, 0.1 mL	4358293, or equivalent
MicroAmp™ Optical 8-Cap Strips	4323032, or equivalent
Additional consumables and reagents	
Filtered pipette tips	**thermofisher.com/pipettetips**
Nuclease-free reagent tubes for preparing master mixes	MLS
1X Phosphate Buffered Saline (PBS), pH 7.4	MLS
InPouch™ TF Bovine	BioMed Diagnostics 11-1003, 11-1010

[1] Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Isolate DNA from samples

Table 2 Sample handling recommendations

Step or process	Recommendation
Transport/store samples	1. Transport inoculated InPouch™ media to the diagnostic lab, according to the manufacturer's instructions. 2. Incubate samples at 37°C for 24 hours.
Prepare cultured smegma samples	1. Thoroughly mix the InPouch™ contents. 2. Transfer 1 mL from the pouch to a 1.5-mL tube. 3. Vigorously vortex the tube. 4. Use 300 µL of the cultured sample for DNA isolation.
(*Optional*) Prepare pooled cultured smegma samples	Pool up to 5 individual cultured smegma samples as described below.[1] 1. Thoroughly mix the individual cultured smegma sample, and create a 300-µL pool of cultured samples by combining equal volumes of individual samples. For example, transfer 60 µL of 5 individual samples to create one pool. 2. Briefly vortex the pooled cultures (properly sealed to prevent cross-contamination) to ensure sufficient mixing, and centrifuge briefly to collect the contents in the lower portion of the tube/plate. 3. Use 300 µL of the pooled smegma samples for each DNA purification, and process in the same way as individual samples.
Prepare mock-purified samples (for use in extraction control PCRs)	Prepare duplicate mock-purified samples, using 1X PBS as the starting material. Process mock-purified samples with the same DNA isolation method that is used for test samples.
Proposed DNA isolation method	5X MagMAX™ Pathogen RNA/DNA Kit (Cat. No. 4462359), or an equivalent DNA isolation method.
Required modifications to the DNA isolation method	• Add 2 µL of undiluted Xeno™ DNA Control (20,000 copies) per purification to the lysis solution used for DNA purification. • Add carrier RNA to the lysis solution according to the manufacturer's recommendation. Add carrier RNA in addition to Xeno™ DNA Control. Carrier RNA is provided in the 5X MagMAX™ Pathogen RNA/DNA Kit.

[1] Pool size is determined by the testing laboratory.

Perform real-time PCR

1 Determine the quantity of reactions and thaw the reagents

a. Plan to include the following control reactions on each plate:
   - Positive control (prepare duplicate reactions); use 8 µL of T. foetus-Xeno™ Control DNA Mix (1,000 copies/µL) per reaction.
   - No-template control (NTC) (prepare duplicate reactions); use 8 µL of Nuclease-free Water in place of sample DNA.

b. Plan the plate layout so that the wells containing NTCs are located as far as possible from positive controls and test samples to prevent accidental cross-contamination.

c. Thaw PCR master mix reagents in one ice bucket and controls and samples in a separate ice bucket. Gently vortex each tube to mix the contents thoroughly, then briefly centrifuge to collect the solution at the bottom of the tube. Keep the reagents on ice.

2 Prepare the real-time PCR master mix on ice

Combine the following components for the number of reactions required plus 10% overage.

Component	Volume per 25 µL reaction
2X qPCR Master Mix	12.5 µL
T. foetus Primer Probe Mix	1.0 µL
Nuclease-free Water	3.5 µL
Total volume of real-time PCR master mix	**17.0 µL**

3 Set up the PCR reactions

a. Dispense 17 µL of real-time PCR master mix to the appropriate wells of a PCR plate or PCR tubes on ice.

b. Add the appropriate component for each reaction type, according to the following table:

Reaction type	Component	Volume per reaction
Test sample	Sample DNA	8.0 µL
NTC	Nuclease-free Water	8.0 µL
Positive control	T. foetus-Xeno™ Control DNA Mix (1,000 copies/µL)	8.0 µL
Extraction control	Mock-purified 1X PBS sample	8.0 µL

c. Seal each reaction vessel, mix, then centrifuge briefly to bring the contents to the bottom.

4 Set up and run the real-time PCR instrument

For detailed information to set up and run the instrument, see the appropriate documentation for your instrument.

a. Following the manufacturer's instructions, set up the run using the following parameters:
   - Experiment type: Standard curve
   - Run mode: Standard
   - Reaction volume: 25 µL
   - ROX™ passive reference dye: Included in the 2X qPCR Master Mix
   - TaqMan® probe reporter dyes and quenchers:

4 Set up and run the real-time PCR instrument *(continued)*

Target	Reporter	Quencher
T. foetus DNA	FAM™ dye[1]	Eclipse™ Q
Xeno™ DNA Control	VIC™ dye[2]	Eclipse™ Q

[1] Absorbance maximum of 495 nm; emission maximum of 520 nm.

[2] Absorbance maximum of 540 nm; emission maximum of 552 nm.

b. Run the thermal cycler program and collect real-time amplification data during stage 2. Use the following thermal cycler settings:

Stage		Reps.	Temp.	Time
Initial denaturation	1	1	95 °C	10 minutes
Amplification	2	40	95 °C 55 °C	15 seconds 45 seconds

Data analysis

See your real-time PCR instrument user guide for instructions on how to analyze your data, using the following method.

Table 3 Data analysis

Method	Details
Use the **Control-Based Threshold** setting for data analysis.	1. Select **Manual CT**. 2. Export ΔR_n values for the positive control samples (T. foetus-Xeno™ Control DNA Mix). 3. Average the FAM™ and VIC™ dye values (separately) for the ΔR_n at cycle 40 for all replicates of the positive control reaction. 4. Set the threshold for the *T. foetus* (Trich) DNA target reactions at 10% of the average maximum fluorescence value of the *T. foetus* amplification signal in the positive control reactions (containing approximately 8,000 copies of T. foetus-Xeno™ Control DNA Mix per reaction). Example: If the average maximum fluorescence value for the *T. foetus* target in the positive control reactions is 0.15, set the *T. foetus* threshold at 0.015. 5. Repeat step 4 for the Xeno™ DNA Control target using a 10% threshold. Example: If the average maximum fluorescence value for the Xeno™ DNA Control target in the positive control reactions is 2.0, set the Xeno™ DNA Control threshold at 0.2.
Check the raw fluorescence data.	Verify that increased fluorescence seen in the normalized data is also evident without mathematical data processing.

Interpretation of test results

Verify that your real-time PCR run is valid before analyzing test sample results.

Table 4 Criteria for a valid real-time PCR run

Reaction type	C_t value for *T. foetus* DNA	C_t value for Xeno™ DNA Control
Positive control	25–32	25–31
NTC	40 (no signal detected)[1]	40 (no signal detected)[1]
Extraction control	40 (no signal detected)[1]	27–34

[1] The run is invalid if the C_t values for either *T. foetus* or Xeno™ DNA Control targets in the NTC are <40. See "Troubleshooting" on page 5.

Table 5 Interpretation of test results from individual samples

C_t value for *T. foetus* DNA	C_t value for Xeno™ DNA Control	Interpretation
<38	⩽40[1]	*T. foetus*-positive sample
40 (no signal detected)	Xeno™ DNA Control C_t shift ⩽2.5[2]	*T. foetus*-negative sample
⩾38	⩽40[2]	Suspect result[2]

[1] High levels of *T. foetus* DNA in the sample can reduce the signal from the Xeno™ DNA Control, resulting in a higher C_t value for Xeno™ DNA Control in *T. foetus*-positive samples. See "Troubleshooting" on page 5.

[2] See Table 6.

Table 6 Assessment of suspect results (for individual samples only)

Result	Action	
Suspect result 1: The sample *T. foetus* C_t value is <40 and the C_t value for one or more NTCs or extraction controls is <40.	Repeat the extraction or real-time PCR. See "No template control reaction:" on page 5.	
Suspect result 2: A *T. foetus* (TF) sample is considered suspect if the TF C_t value is 38–40.	Analyze suspect DNA samples for the presence/absence of PCR inhibitors by calculating the Xeno™ DNA Control C_t Shift: **Xeno™ DNA Control C_t Shift** = **SS** – **XEC**, where: **SS** = C_t of Xeno™ DNA Control in the suspect sample **XEC** = Average C_t of Xeno™ DNA Control in the extraction controls	
	Workflow A **Xeno™ DNA Control C_t shift <2.5**	**Workflow B** **Xeno™ DNA Control C_t shift ⩾2.5**
	1. Repeat the DNA purification on triplicate aliquots of the original diagnostic sample. 2. Repeat the real-time PCR with 8 µL of purified DNA from step 1. 3. Determine the number of samples with a C_t value <40: • 0 of 3: *T. foetus* negative • 1 of 3: Presumptive positive • ⩾2 of 3: *T. foetus* positive	1. Repeat the real-time PCR with 2 µL of the suspect DNA sample. (PCR inhibitors may be present in the DNA.) If the *T. foetus* C_t value is: • <38: The sample is *T. foetus* positive. No further testing is required.[1] • ⩾38: Continue with steps 2–5 of this workflow. 2. Dilute the original individual diagnostic sample 1:4 with 1X PBS. 3. Repeat the DNA purification on triplicate aliquots of the 1:4 diluted sample. 4. Repeat the real-time PCR with 8 µL of the purified DNA from step 3. 5. Determine the number of samples with a *T. foetus* C_t value <40: • 0 of 3: *T. foetus* negative • 1 of 3: Presumptive positive • ⩾2 of 3: *T. foetus* positive

[1] The Xeno™ DNA Control C_t value on diluted samples is not considered utilizing this workflow.

Table 7 Interpretation of test results from pooled samples

C_t value for *T. foetus* DNA	C_t value for Xeno™ DNA Control	Interpretation	Action
<40 (signal detected)	<40 (signal detected)	*T. foetus*-positive pool	Retest individual samples from the positive pool to determine which individual sample or samples caused the positive result. Interpret the retest results according to Table 5.
40 (no signal detected)	Xeno™ DNA Control C_t shift ⩽2.5	*T. foetus*-negative pool	None required
	Xeno™ DNA Control C_t shift ⩾2.5	Suspect result	Repeat the DNA purification with the individual cultured smegma samples, then repeat the real-time PCR with each DNA sample. Interpret the retest results according to Table 5.

Troubleshooting

Observation	Possible cause	Recommended action
Positive control reaction: *T. foetus* DNA—no signal Xeno™ DNA Control—no signal	The T. foetus-Xeno™ Control DNA Mix was improperly handled, resulting in DNA degradation.	Use appropriate precautions against DNase contamination when handling the control DNAs. For example, wear clean gloves and use nuclease-free barrier pipette tips.
	The 2X qPCR Master Mix was stored or handled improperly and it lost activity.	Repeat the real-time PCR with fresh reagents.
	The thermal cycler was not properly set up.	Check the thermal cycler settings. See "Set up and run the real-time PCR instrument" on page 2.
	The real-time PCR master mix was prepared incorrectly.	Repeat the test with correctly prepared real-time PCR master mix.
No template control reaction: Signal detected (C_t value is <40)	There was contamination during the PCR.	• Repeat the real-time PCR with fresh reagents and freshly decontaminated pipettes. • Set up the real-time PCR in an area separate from areas used for DNA isolation and PCR product analysis.
Extraction control reaction: Signal detected (C_t value is <40)	There was contamination during the DNA isolation or PCR.	• Repeat the DNA isolation or real-time PCR with fresh reagents and freshly decontaminated pipettes. • Set up the real-time PCR in an area separate from areas used for DNA isolation and PCR product analysis.
Test samples Xeno™ DNA Control—no or low signal *T. foetus* DNA—high signal	The Xeno™ DNA Control primers and probe are at limiting concentrations in the real-time PCR. High levels of *T. foetus* DNA in a sample can reduce amplification of the Xeno™ DNA Control.	No or low signal from Xeno™ DNA Control is expected in a reaction that has a strong signal for *T. foetus* DNA.
Test samples: Xeno™ DNA Control—no signal *T. foetus* DNA—no signal or suspect-range signal	Poor DNA recovery.	• Check the C_t values of Xeno™ DNA Control in the mock-purified samples. • A C_t value ≥38 in individual or pooled samples indicates that Xeno™ DNA Control was omitted or that DNA recovery was poor. • Repeat the DNA purification of the original diagnostic sample.
	The DNA samples contain PCR inhibitors.	See Table 6.

Explanation of symbols

The symbols present on the product label are explained in the following table.

	MANUFACTURER		USE BY
REF	CATALOG NUMBER		CONSULT INSTRUCTIONS FOR USE
LOT	BATCH CODE		CAUTION
SN	SERIAL NUMBER		TEMPERATURE LIMIT

Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-PCR amplification:

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

Documentation and support

Customer and technical support

In the United States, call 1-800-955-6288.

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Product documentation

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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Revision history: Pub. No. 4485468

Revision	Date	Description
B	2 August 2018	• Updated the list of compatible real-time PCR systems. • Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
A	18 September 2013	Baseline for this revision history.

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