Improved detection of *T. foetus* by RT-qPCR eliminates the need for culture medium

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Introduction

Tritrichomonas foetus (*T. foetus*) is a protozoan that is the causative agent of bovine trichomoniasis, a sexually transmitted disease found worldwide in bulls and cows. The previous DNA-based Trich detection kit, a quantitative PCR (qPCR) assay, has been considered the gold standard for PCR-based detection of *T. foetus* in preputial samples (smegma) collected in culture medium. However, in 2018, the Texas Veterinary Medical Diagnostic Laboratory (TVMDL) published primers and probe sequences that target the 5.8S ribosomal RNA rather than the gene, which generates earlier C_t values due to higher concentration of target template when compared to the DNA-targeting qPCR test. Since combining the reverse-transcription qPCR (RT-qPCR) primers and probe design with a one-step RT master mix makes the reaction more sensitive, it eliminates the need to collect and incubate smegma in the InPouch™ commercial culture medium and offers a simple PBS sample collection instead. Additionally, due to the higher target template concentration and the reduction in inhibition, it provides the capability to pool several smegma samples for nucleic acid extraction and RT-qPCR.

TVMDL RT-qPCR reagents vs. Applied Biosystems™ *T. foetus* Reagents TAMUC

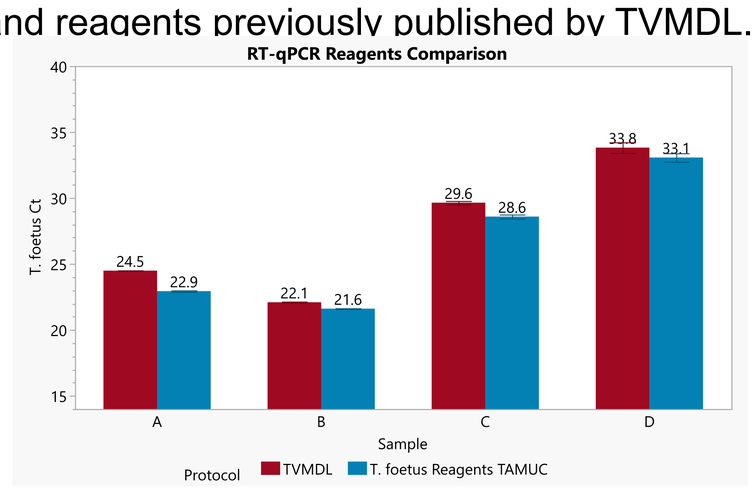
Methodology

Isolated nucleic acid was subjected to RT-qPCR, in triplicate, following either the protocol published by TVMDL in Ginter Summarell et al. [1], or a protocol incorporating the Applied Biosystems™ *T. foetus* Reagents TAMUC (Texas A&M University Custom).

TVMDL RT-qPCR reagents	*T. foetus* Reagents TAMUC
TVMDL primers and probe design	TVMDL primers and probe design
Applied Biosystems™ Path-ID™ qPCR Master Mix Buffer	Applied Biosystems™ TaqMan® Fast Virus 1-Step Master Mix
Applied Biosystems™ ArrayScript™ RT	(RT enzyme in master mix)

Results

T. foetus Reagents TAMUC demonstrated better performance when compared to the protocol and reagents previously published by TVMDL.



Complete workflow comparison

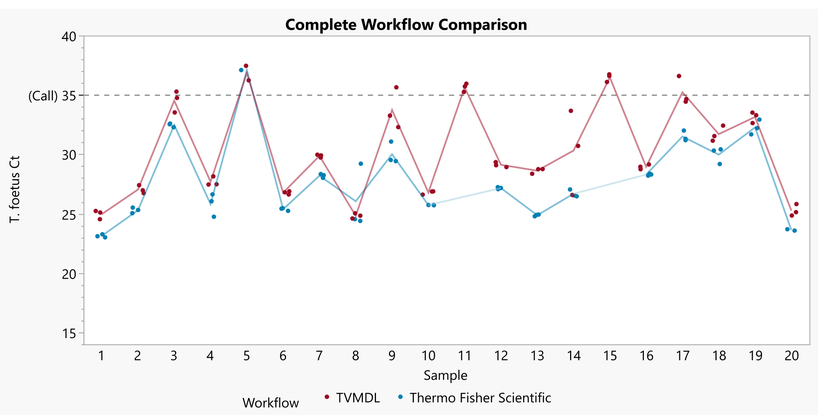
Methodology

Two different complete workflows were used to isolate nucleic acid, in triplicate, from 20 positive and 3 negative smegma samples. The isolated nucleic acid was then assessed for the presence of *T. foetus* by RT-qPCR with the published TVMDL RT-qPCR reagents or the *T. foetus* Reagents TAMUC.

TVMDL workflow	Thermo Fisher Scientific workflow
TVMDL sample prep	Applied Biosystems™ MagMAX™ CORE Nucleic Acid Purification Kit
TVMDL RT-qPCR reagents	Applied Biosystems™ *T. foetus* Reagents TAMUC

Results

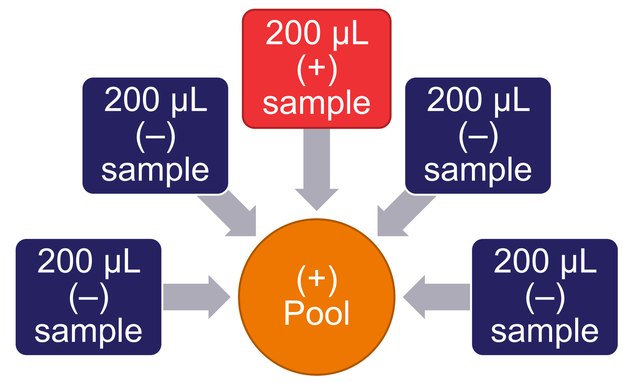
When compared to the complete workflow by TVMDL, the complete workflow by Thermo Fisher Scientific yielded earlier C_t values overall, and all calls were identical.



Pooling study

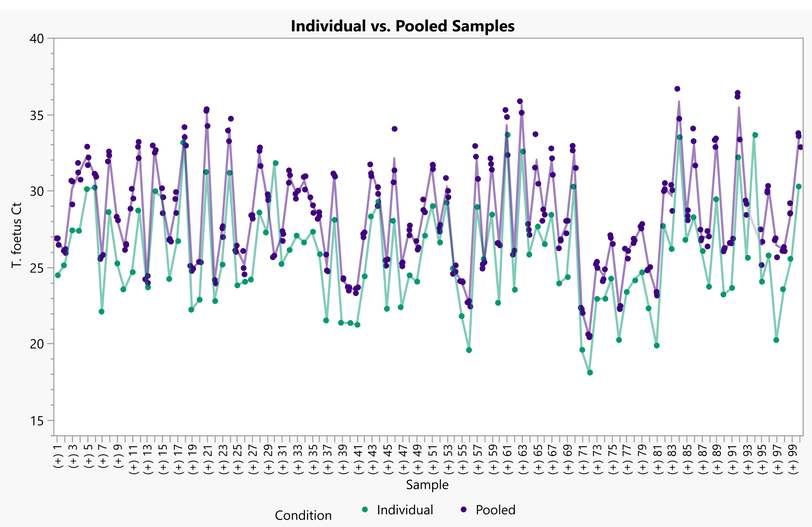
Methodology

TVMDL used *T. foetus* Reagents TAMUC to test 100 positive and 100 negative pools, in triplicate, consisting of 1 positive or negative sample combined with 4 negative samples.



Results

When using the *T. foetus* Reagents TAMUC to test samples in pools of five, the proposed reagents provided equivalent calls to those obtained by testing the samples individually, with an efficiency of 91±5%.



Conclusion

In collaboration with TVMDL, several individual and pooled smegma samples were tested using both TVMDL published and TAMUC reagents and all calls were identical. The incorporation of TaqMan Fast Virus 1-Step Master Mix with TVMDL's primers and probe design results in a protocol with significantly better performance when compared to the TVMDL-published protocol and the previous DNA-based Trich detection kit.

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Reference

Ginter Summarell C et al. (2018) Improvements in *Tritrichomonas foetus* molecular testing. *Journal of Veterinary Diagnostic Investigation* 30:603–608.

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