AMD Ltd *Trichomonas vaginalis* qPCR Detection Kit

CE IVD

REF **KD919213-100**

Advanced Molecular Diagnostics Ltd is a diagnostics company specialising in the manufacture and supply of molecular biology instruments, reagents and consumables.

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Intended Use

The assay is an *in vitro* Real-Time PCR assay for the qualitative detection of ***T. vaginalis*** **in male urethral or endocervical canal swab samples** using the hydrolysis probe detection method in a highly sensitive one step qPCR kit.
For use in i*n vitro* Diagnostics.

Overview

T. vaginalis is a sexually-transmitted parasite that causes the disease trichomoniasis. This infection can affect both men and women in equal measure, but usually male patients are asymptomatic.[1] Symptoms in woman can include vaginitis (vaginal inflammation).[2]
As with many sexually-transmitted infections, several complications can occur if this infection is left untreated during pregnancy.

Principle of the Test

This kit is designed for the detection of *T. vaginalis* in male urethral or endocervical canal swab samples using hydrolysis probe qPCR. Amplification of the unique sequence, which is labelled with fluorescent reporter dyes, is followed by detection by the hydrolysis probe method of qPCR.
As the new target DNA strand is synthesized, the tightly bound probe is cleaved by the 5' to 3' exonuclease activity of Taq polymerase, which releases the fluorescent reporter from the quencher and substantially increases the fluorescent signal. The point at which the fluorescence becomes detectable above the background, the quantification cycle (Cq), is proportional to the amount of target present in the sample. The lower the Cq, the greater the amount of target present.
This assay consists of a forward primer, a reverse primer and a probe labelled with a 5' **FAM™** reporter dye and a 3' quencher, and *T. vaginalis* presence is indicated by the **FAM** fluorophore. An internal positive control assay is also provided in order to assess the quality of the extracted DNA and the effect of any PCR inhibitors that may be present. These assays are also multiplexed in a ready-to-use PCR master mix which utilises hot start technology, thus minimising non-specific reactions and ensuring maximum sensitivity. It also contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR reaction by amplification products.

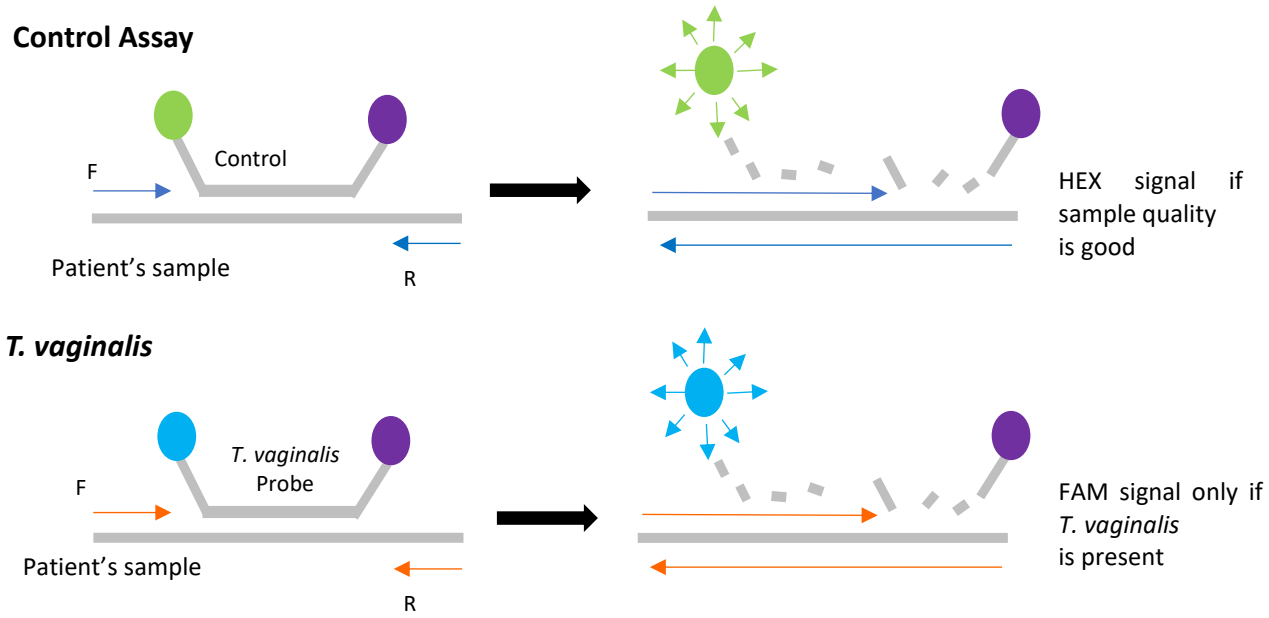

Figure 1. The principle of qPCR with hydrolysis probe detection for identifying the presence of T. vaginalis in male urethral or endocervical canal swab samples. The control assay in the master mix will produce a HEX signal if the DNA quality is acceptable. The T. vaginalis assay will produce a FAM signal, if T. vaginalis is present, however if it is not present, no FAM signal will be detected. Due to assay competition, the HEX signal may be reduced or absent when the FAM signal is strong.

Materials Provided

Kit Contents

Item	
***T. vaginalis* qPCR one step M. Mix**	2 x 1 ml
***T. vaginalis* Positive Control**	1 x 0.05 ml
Nuclease-free Water	1 x 1 ml

Reagent Storage and Handling

The kits should be transported and stored at temperatures between -15°C and -25°C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept. Repeated freezing and thawing of the kit components may result in lower detection quality. It is recommended that the master mix is aliquoted to avoid this. Avoid exposure to light. Ensure that all reagents are thoroughly thawed, mixed and pulse centrifuged before use.

Materials and Equipment Required (not provided)

DNA Extraction: Leading brands of IVD DNA extraction kit are acceptable for use with this diagnostic kit. If using any other kit, please validate for use with this assay before proceeding with sample testing.

PCR Instrument: This kit should be used with qPCR systems which can detect FAM and HEX fluorescent dyes. It is also compatible with low, high and no ROX instruments.

Consumables: AMD manufactures high quality nuclease and pyrogen free PCR plastic ware suitable for use with this kit. Use of other manufacturers' consumables is also acceptable.

Other Laboratory Equipment: Vortex, micro centrifuge, micro pipettes and tips, microfuge tube rack, PCR tube/plate rack, spectrophotometer.

Warnings and Precautions

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). Discard sample and assay waste according to your local safety regulations. It is essential to follow the instructions in this manual precisely, to ensure accurate results. Please familiarise yourself with this product manual and your qPCR instrument before using the AMD Zena Max *T. vaginalis* qPCR Kit.

Sample collection, Storage and Transport

The sample for AMD *T. vaginalis* qPCR Kit should be collected from swabs. Specimen collection swabs for *T. vaginalis* culture must have a plastic or wire shaft and either rayon, dacron, or cytobrush tip. Other materials might inhibit isolation. Specimen collection for *T. vaginalis* culture is invasive, requiring the insertion of a swab 2–3 cm into the male urethral or 1–2 cm into the endocervical canal followed by two or three rotations to collect sufficient columnar or cuboidal epithelial cells, according to the CDC. Please ensure that the sample is stored correctly and kept away from any contamination. Aliquot and store the samples at -20^{0}C or -80^{0}C immediately if they are not to be used within this time-period. Freeze thawing may compromise the test results. Ensure that samples are stored correctly and kept away from any contamination.

For transportation, the samples should be placed in a shatterproof transport container to avoid the potential danger of infection due to sample leakage. Transport samples following the local and national instructions for the transport of pathogenic material, by courier, if possible, at a temperature of 2-8^{0}C and no longer than 6 hours following collection.

Assay Procedure

Sample preparation

For optimal results use AMD LUCO Total DNA Extraction Kit to elute the DNA from the sample. It is important to ensure that all samples are kept free from any contamination and correct storage procedures are followed to ensure there is no damage to the DNA. Store the DNA at 2-8°C for up to 24 hours, then at -20°C for longer term storage to ensure there is no damage to the DNA.

PCR Set Up

1. Ensure that all reagents and samples are thawed completely, mixed and briefly centrifuged. Keep all reagents and samples on ice during this procedure.
2. Set up the reactions using the table below.

Product	Volume X1
***T. vaginalis* M. Mix**	20µl
DNA Sample/control	5µl

3. Add the DNA samples and standards to the PCR tubes/plate. Also add 5 µl nuclease free water in place of the DNA as a No Template Control (NTC).

4. Seal the PCR tubes or plate and briefly spin to ensure that the reagents are at the bottom and no air bubbles are present.
5. Place the plate/tubes in the qPCR thermal cycler and use the following thermal profile:

Thermal Profile: set the qPCR instrument to the stages below

Stage/Step	Temperature	Time
Stage 1: Step 1	30°C	2mins
Stage 1: Step 2	95°C	2mins
40 Cycles		
Stage 2: Step 1	95°C	10secs
Stage 2: Step 2	58°C	30secs

*Data collection step in FAM (diagnostic assay) and HEX (internal control assay) channels.

6. When the run has finished, dispose of the PCR reaction tubes/plate in an appropriate manner in accordance with local and national regulations.

Data Analysis

Analyse the data if the software does not do this automatically at the end of the run. Export the data to Excel or a PDF report, depending on the qPCR instrument used, and view the results.

Interpretation of Results

The results of this qualitative assay should be interpreted as follows, using Table 1 as a quick reference guide:

- The internal control assay signal in the HEX (yellow) channel should be present but may be absent or have a high Cq value (low signal) when the diagnostic assay (FAM) signal is strong.
- If there is a signal in the FAM (green) channel, with or without a HEX signal, the sample is **positive** for T. vaginalis.
- If there is a HEX signal but no FAM signal, the sample is **negative** for T. vaginalis.
- If there is no signal in either channel, the result is **inconclusive**.

Result		Interpretation
HEX (IC)	**FAM (T. vaginalis)**	
Positive	Positive (Ct<38)	Positive for *T. vaginalis*
No Cq	Positive (Ct<38)	Positive for *T. vaginalis*
Positive (Ct<34)	No Cq	Negative for *T. vaginalis*
No Cq	No Cq	Inconclusive

Table 1. Interpretation of the results obtained from the Zena Max *T. vaginalis* qPCR Kit.

Technical Specifications

Quality: All AMD kits are manufactured under high quality standard methods and unique precision.
Sensitivity: AMD *T. vaginalis* kits are very sensitive, reaching up to 10 copy/rxn "rxn volume 25µl" under our validation methods and devices.
Specificity: AMD *T. vaginalis* are very specific with up to 100% under our validation methods and devices.

Product Limitations

The kit is for *in vitro* diagnostic procedures and should only be used by specifically trained laboratory personnel. The expiry date of all components must be checked before use and disposed of if expired. Occasionally mutations may arise in the genomic region targeted by the primers and probes of this this assay, leading to reduction in performance or failure of the assay. The assay design and efficacy are reviewed periodically.

Additional Information

AMD produces real-time PCR kits with a wide range of applications for researchers from gene expression analysis, cDNA and population genotyping studies to the multiplex detection of several disease targets real-time PCR with excellent sensitivity and specificity.

References

1. Hook E. W., 3rd (1999). Trichomonas vaginalis--no longer a minor STD. *Sexually transmitted diseases*, *26*(7), 388–389. https://doi.org/10.1097/00007435-199908000-00004
2. Nanda, N., Michel, R. G., Kurdgelashvili, G., & Wendel, K. A. (2006). Trichomoniasis and its treatment. *Expert review of anti-infective therapy*, *4*(1), 125–135. https://doi.org/10.1586/14787210.4.1.125

Contact

If you have any queries, comments or complaints please refer to our website at:

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Harmonised Symbols

The following is a key of all harmonised symbols used by AMD Ltd (Advanced Molecular Diagnostics) in Instructions for Use (IFUs) and product labelling.

Symbol	Definition	Details
	Manufacturer name and address	AMD Ltd BioCity Nottingham, Pennyfoot Street, Nottingham NG1 1GF United Kingdom
	UDI-DI number for the product given	Basic: 506105998TVAGEP UDI-DI: (01)05061059980793 UDI-PI: See label
	Minimum and maximum storage temperatures for this product	-15 to -25 degrees Celsius
	Catalogue number	KD919213-100
	Number of tests/reactions in this pack	100
	CE-IVD certified	According to Directive 98/79/EC