AMD Ltd Zena Max Gardnerella vaginalis qPCR Detection Kit

CE IVD

REF **KD654308-100**

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* PCR reaction assay for the qualitative determination of *G. vaginalis* DNA in a human sample such as sputum and bronchoalveolar lavage (BAL) based on the hydrolysis probe detection method for Human *G. vaginalis* with a highly sensitive one step qPCR kit.

For use in *in vitro* diagnostics.

Overview

Gardnerella vaginalis is a small, facultatively anaerobic, Gram-variable bacterium that is commonly associated with bacterial vaginosis (BV), a condition characterized by a disruption of the normal vaginal microbiota. While it can be present in healthy individuals, overgrowth of G. vaginalis, often alongside other anaerobes—leads to a decrease in protective Lactobacillus species and an increase in vaginal pH. This imbalance can cause symptoms such as thin, greyish discharge and a characteristic fishy odor, although many cases remain asymptomatic. Transmission is not strictly sexual, but sexual activity can influence its occurrence. Diagnosis is typically made using Amsel's criteria or Nugent scoring from vaginal swabs, and confirmation can involve molecular tests like PCR. Treatment generally involves antibiotics such as metronidazole or clindamycin, though recurrence is common due to persistence of biofilms and incomplete restoration of normal flora.

Principle of the Test

The qPCR kit is designed for the detection of *Gardnerella vaginalis* by the real-time Polymerase Chain Reaction (PCR) method. Detection is based on the amplification of a specific conservative DNA sequence for the gene M181 that encodes for a unique CARD5 virulence factor. Then measuring the amplification product concentration using PCR process and fluorophore labelled probes. *G. vaginalis* presence is indicated by the FAM fluorophore fluorescence growth. For the DNA isolation quality control and possible PCR inhibition control there are primers and probe for internal control gene amplification present in the reaction mix. Amplification of internal control gene is indicated in the HEX fluorophore fluorescence channel. The detection kit utilizes the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity. The ready-to-use Master Mix contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR reaction by amplification products. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

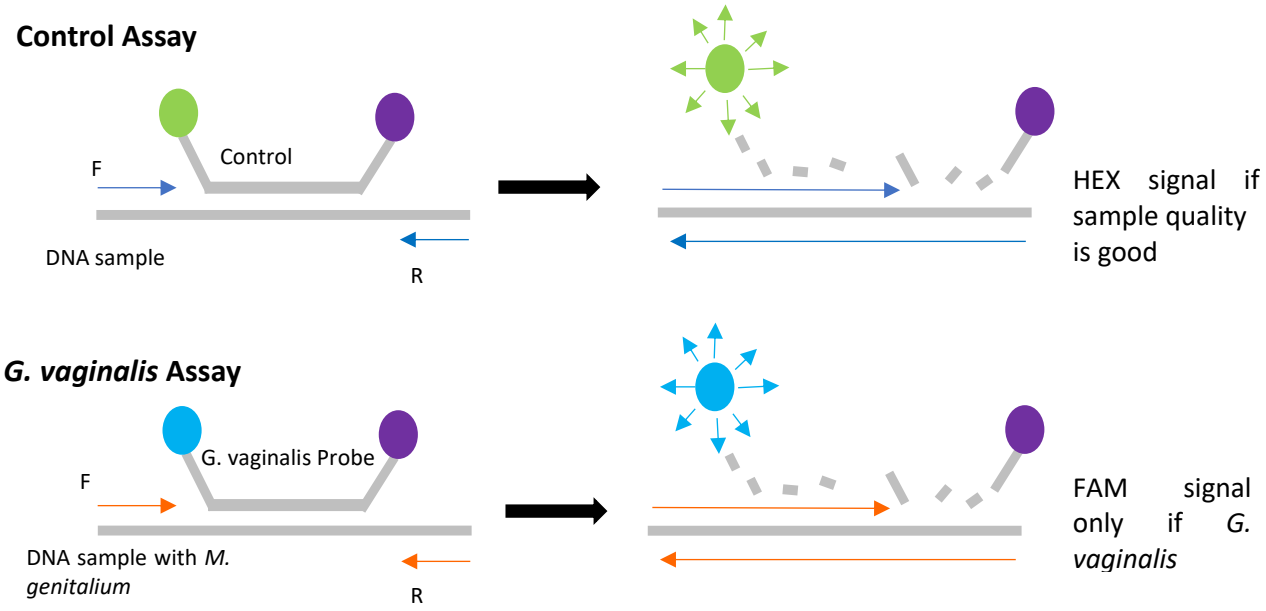

Figure 1. The principle of qPCR with hydrolysis probe detection for identifying the presence of G. vaginalis. The control assay in the master mix will produce a HEX signal if the DNA quality is acceptable. The *G. vaginalis* assay will produce a FAM signal, if *G. vaginalis* is present, however if it 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 Content

Product	Volume
***G. vaginalis* PCR M. Mix**	2 x 1 ml
***G. 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 freeze 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: AMD manufactures the LUCO AMD DNA Extraction Kit which can be used for extracting DNA from the samples. Other 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 both low and high 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 G. vaginalis qPCR Kit.

Sample collection, Storage and Transport

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 DNA Extraction Kit to elute the DNA from the bronchoalveolar lavage, sputum or nasal mucus 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
***G. vaginalis* PCR M. Mix**	20µl
DNA Sample	5µl

3. Add the DNA samples and Control to the PCR tubes/plate. Also add 5µl 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

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

*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

This is a qualitative assay which indicates the presence or absence of G. vaginalis. The results should be interpreted as follows, using Table one 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 G. vaginalis and the amount present in the original sample can be calculated using the equation below.
- If there is a HEX signal but no FAM signal, the sample is **negative** for G. vaginalis.
- If there is no signal in either channel, the result is **inconclusive**.

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

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

Technical Specifications

Quality: All AMD kits are manufactured under high quality standardization methods and unique precision and sensitive technology study compared by most of famous and approved diagnostic commercial *G. vaginalis* assays.

Sensitivity: AMD Gardnerella vaginalis kit is very sensitive kit reaches up to 10 copies per "rxn volume 25µl" under our validation methods and devices.

Specificity: AMD Gardnerella vaginalis kit is very specific up to 100% and targeting *G. vaginalis* M181 gene under our validation methods and devices.

Product Limitations

This 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 region of the genome targeted by the primers and probes of this assay, leading to under-quantification or failure to detect the presence of the pathogen in these cases. Assay design and efficacy is 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. Please familiarise yourself with the qPCR instrument before using the AMD *G. vaginalis* detection kit.

Contact

If you have any queries, comments or complaints please refer to our website at:
www.am-diagnostics.co.uk
info@am-diagnostics.co.uk

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: 506105998MPNEUDB UDI-DI: (01)05061059980373 UDI-PI: See label
	Minimum and maximum storage temperatures for this product	-18 to -25 degrees Celsius
	Catalogue number	KD654308-100
	Number of tests/reactions in this pack	100
	CE-IVD certified	According to Directive 98/79/EC