



AMD Ltd Zena Max *Treponema pallidum* qPCR Detection Kit

CE

IVD

REF

KD5253602-100

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

info@am-diagnostics.co.uk

+44(0)115 969 9934



AMD Ltd
BioCity Nottingham
Pennyfoot Street
Nottingham
NG1 1GF
United Kingdom



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Intended Use

The assay is an *in vitro* PCR reaction assay for the qualitative determination of *T. pallidum* in human samples based on the hydrolysis probe detection method.

For use in *in vitro* diagnostics.

Overview

Treponema pallidum, also known as *Spirochaeta pallida*, is a gram-negative bacterium with a subspecies that causes syphilis, bejel and yaws. It is transmitted via humans or baboons through entering the mucosal membranes or open lesions in the skin. Although the transmission of this bacterium primarily occurs through sexual contact, it can occur from transmission from mother to foetus. Transmission of this bacterium can also increase the likelihood of HIV transmission.

Principle of the Test

The qPCR kit is designed for the detection of *T. pallidum*. Detection is based on the amplification of a specific conservative DNA sequence within the *T. pallidum* genome. Its presence is indicated by 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.

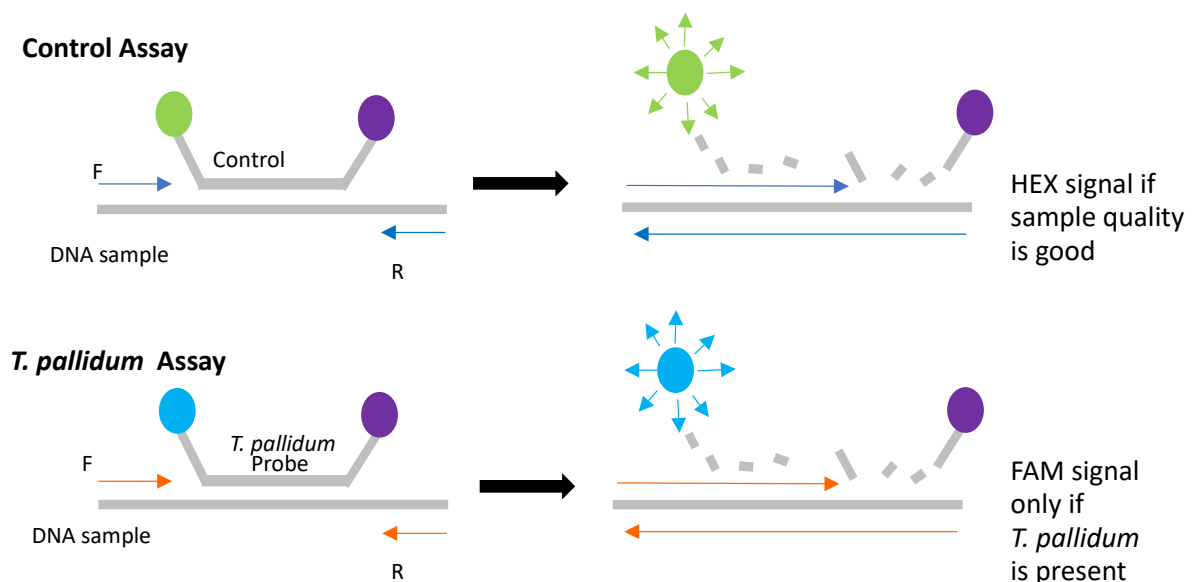


Figure 1. The principle of qPCR with hydrolysis probe detection for identifying the presence of *T. pallidum*. The control assay in the master mix will produce a HEX signal if the DNA quality is acceptable.



The *T. pallidum* assay will produce a FAM signal, if *T. pallidum* 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

Product	Volume
<i>T. pallidum</i> PCR M. Mix	2 x 1 ml
<i>T. pallidum</i> 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 IVD 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 *T. pallidum* qPCR Kit.



Sample collection, Storage and Transport

Aliquot and store the samples at -20°C or -80°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°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 no DNA damage.

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 on a cool block or ice using the table below, ensuring to include duplicate reactions for all samples and controls.

Product	Volume
<i>T. pallidum</i> PCR M.Mix	20µl
PCR Setup 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	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 appropriately 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 *T. pallidum*. 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 *T. pallidum* 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 *T. pallidum*.
- If there is no signal in either channel, the result is **inconclusive**.

Result		Interpretation
HEX	FAM	
Positive	Positive (Ct<38)	Positive for <i>T. pallidum</i>
No Cq	Positive (Ct<38)	Positive for <i>T. pallidum</i>
Positive (Ct<34)	No Cq	Negative for <i>T. pallidum</i>
No Cq	No Cq	Inconclusive

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

Technical Specifications

Quality: All Advanced Molecular Diagnostics kits are manufactured under high quality standardization methods and unique precision and sensitive technology study compared by most of famous and approved diagnostic commercial *T. pallidum* assays.

Sensitivity: AMD *T. pallidum* kit is very sensitive kit reaches up to 10 copies per “rxn volume 25µl” under our validation methods and devices.

Specificity: AMD *T. pallidum* kit is very specific up to 100% and targeting *T. pallidum* 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 virus 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 *T. pallidum* detection kit.

References

1. Spencer, RC. *T. pallidum* (2003), Journal of Clinical Pathology, 56 (3), 182-187
2. Makino, S., Watarai, HI, Cheun, T et al, *Effect of the lower molecular capsule released from the cell surface of T. pallidum on the pathogenesis of anthrax* (2002), Journal of Infectious Diseases 186, 227-233.


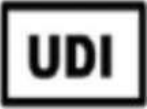




Contact

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: 506105998 UDI-DI: (01)0506105998 UDI-PI: See label
	Minimum and maximum storage temperatures for this product	-15 to -25 degrees Celsius
	Catalogue number	KD5253602-100
	Number of tests/reactions in this pack	100
	CE-IVD certified	According to Directive 98/79/EC