AMD Ltd Zena Max U. parvum, U. urealyticum and M. hominis Multiplex qPCR Detection Kit

CE IVD

REF **KD654208M-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 *Ureaplasma parvum (U. parvum), Ureaplasma urealyticum (U. urealyticum) and Mycoplasma hominis (M. hominis)* in male urethral or endocervical canal swab samples using the TaqMan® detection method in a highly sensitive one step qPCR kit.

Overview

Ureaplasma parvum, Ureaplasma urealyticum, and Mycoplasma hominis are small, cell wall–deficient bacteria belonging to the family *Mycoplasmataceae* that commonly colonize the human urogenital tract. While they can be present as part of the normal genital flora, under certain conditions they act as opportunistic pathogens. U. parvum and U. urealyticum are frequently associated with nongonococcal urethritis, bacterial vaginosis, chorioamnionitis, preterm birth, and neonatal infections, with differences mainly in genomic size and pathogenic potential between the two species. M. hominis is linked to bacterial vaginosis, pelvic inflammatory disease, postpartum and post-abortal fever, and occasionally systemic infections such as sepsis in immunocompromised individuals or neonates. Transmission is typically sexual or vertical (mother-to-child during birth). Because they lack cell walls, these organisms are inherently resistant to beta-lactam antibiotics; treatment usually involves tetracyclines, macrolides, or fluoroquinolones depending on susceptibility. Their clinical relevance lies in reproductive health complications, perinatal outcomes, and opportunistic infections in vulnerable hosts.

Principle of the Test

This kit is designed for the detection of *Ureaplasma parvum, Ureaplasma urealyticum, and Mycoplasma hominis* 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.

During PCR amplification, forward and reverse primers hybridize to the target DNA. A fluorogenic probe is included in the same reaction mixture, which consists of an oligonucleotide labelled with a 5'-reporter dye and a downstream 3'-quencher. The probe is hydrolysed by the 5' to 3' exonuclease activity of Taq polymerase, leading to a physical separation of the fluorescent reporter from the quencher (Figure 1). The detector is then able to record this as an incremental increase in fluorescence signal from that well. If the pathogen in question is not present, the signal specific to that gene will not be produced.

The assay consists of pairs of forward and reverse primers, and probes labelled with 5' fluorescent reporter dyes and 3' quenchers. An internal positive control assay is also provided 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.

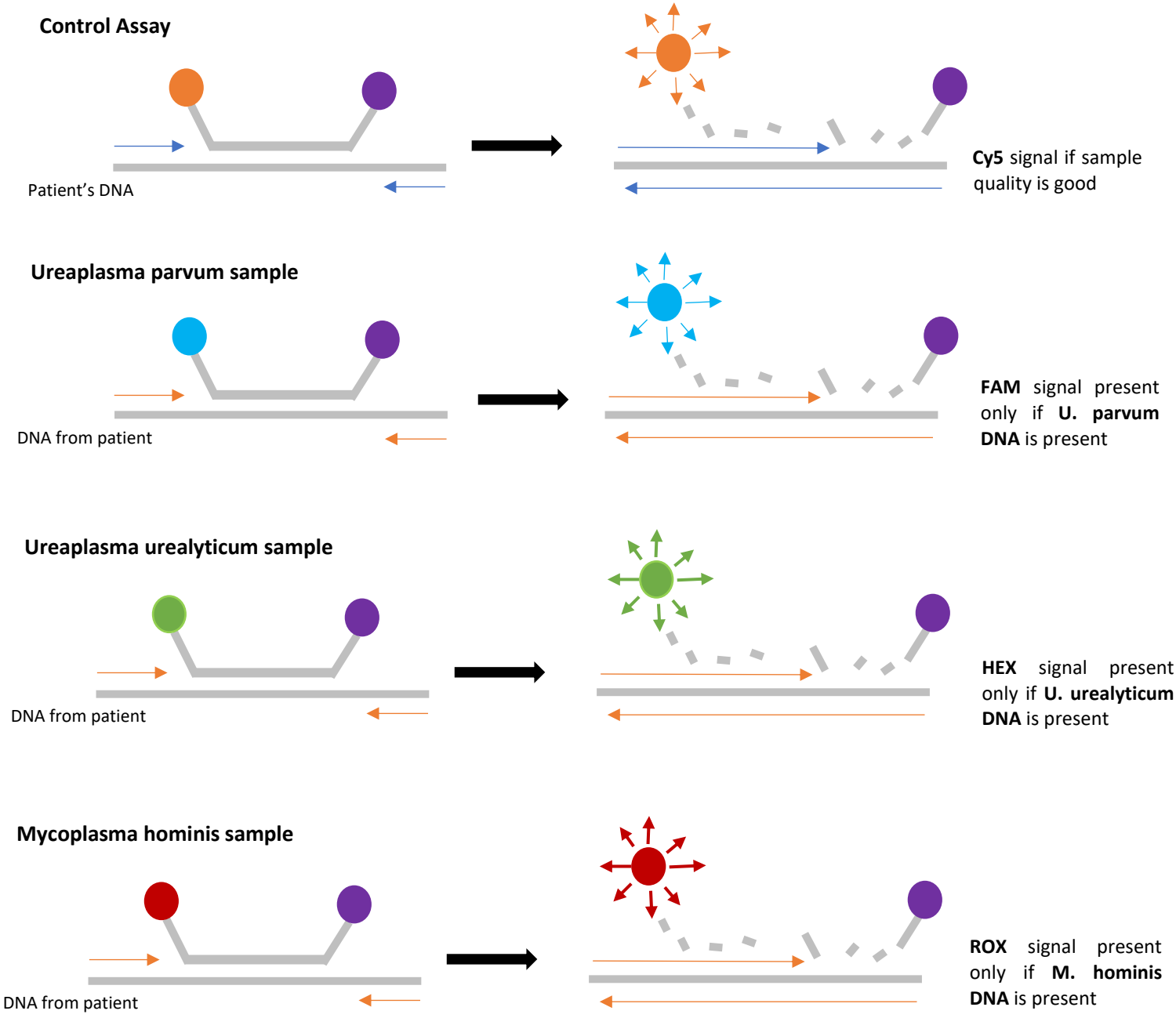

Figure 1. The principle of qPCR with hydrolysis probe detection for identifying the presence of DNA. The control assay in the master mix will produce a CY5 signal if the DNA quality is acceptable. If an STI positive sample is tested, the UP/UR/MH assay will produce a fluorescent signal (FAM, HEX, ROX) to indicate the particular STI DNA present in the patient sample. If no DNA from any of the four STIs detected in this multiplex are present, only the CY5 signal will be detected. Due to assay competition, the CY5 signal may be reduced or absent when other signals are strong.

Materials Provided

Kit Contents

Item	
UP/UR/MH qPCR one step M. Mix	2 x 1 ml
UP/UR/MH 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: For optimal results use AMD LUCO Total DNA Extraction Kit to elute the DNA from the sample. However, any 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, HEX, ROX and Cy5 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. The use of other manufacturers' consumables is also acceptable.

Other Laboratory Equipment: Vortex, microcentrifuge, micropipettes 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 U. parvum, U. urealyticum and M. hominis Multiplex qPCR Kit.

Instrument compatibility

The AMD U. parvum, U. urealyticum and M. hominis Multiplex qPCR kit is compatible with the most common Real Time PCR equipment with the capability of detecting FAM, HEX, ROX, CY5 and CY5.5 (Quasar 705) fluorescent dyes such as Biorad CFX96, Applied Biosystems 7500 Fast, QuantStudio 3,5,7, StepOne Plus, Aglient Mx3000, 3005P, Rotorgene Q, Cepheid Smartcycler, Analytik Jena qTower and Roche Lightcycler 480, 96.

Sample collection, Storage and Transport

The sample for AMD U. parvum, U. urealyticum and M. hominis Multiplex qPCR Kit should be collected from swabs. Specimen collection swabs for *Ureaplasma parvum, Ureaplasma urealyticum, and Mycoplasma hominis* must have a plastic or wire shaft and either rayon, dacron, or cytobrush tip. Other materials might inhibit isolation. Specimen collection for *C. trachomatis* and *N. gonorrhoeae* 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**
U. parvum, U. urealyticum and M. hominis Multiplex M. Mix	20 µl
DNA Sample/ control	5 µl

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

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 setting up the sample information in the qPCR software, enter the remark of the controls and define them as a control in order to automatically obtain a result.

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 Cy5 channel should be present but may be absent or have a high Cq value (low signal) when the diagnostic assay signal is strong.
- If there is a signal in any of the diagnostic channels, with or without a Cy5 signal, the sample is **positive** for the corresponding STI (either Ureaplasma parvum, Ureaplasma urealyticum, and Mycoplasma hominis). A coinfection is possible to occur and so a positive result in multiple channels should not be assumed to be a test failure.
- If there is a Cy5 signal but no other signal, the sample is only **negative** for the STIs that this kit tests for.
- If there is no signal in either channel, the result is **inconclusive**.

Cy5 (IC)	**FAM U. parvum**	**HEX U. urealyticum**	**ROX M. hominis**	**Interpretation**
Positive (Ct<34)	No Cq	No Cq	No Cq	Negative for all the tested STDs
No Cq	No Cq	No Cq	No Cq	Inconclusive
Positive	Positive (Ct<38)	No Cq	No Cq	Positive for *U. parvum*
No Cq	Positive (Ct<38)	No Cq	No Cq	Positive for *U. parvum*
Positive	No Cq	Positive (Ct<38)	No Cq	Positive for *U. urealyticum*
No Cq	No Cq	Positive (Ct<38)	No Cq	Positive for *U. urealyticum*
Positive	No Cq	No Cq	Positive (Ct<38)	Positive for *M. hominis*
No Cq	No Cq	No Cq	Positive (Ct<38)	Positive for *M. hominis*

Table 1. Interpretation of the results obtained from the AMD U. parvum, U. urealyticum and M. hominis Multiplex qPCR Kit.

Technical Specifications

Quality: All AMD kits are manufactured under high quality standard methods and unique precision.

Sensitivity: AMD U. parvum, U. urealyticum and M. hominis Multiplex qPCR kits are very sensitive, reaching up to 10 copy/rxn "rxn volume 25µl" under our validation methods and devices.

Specificity: AMD U. parvum, U. urealyticum and M. hominis Multiplex qPCR kits are very specific, with up to 100% for *Ureaplasma parvum, Ureaplasma urealyticum, and Mycoplasma hominis* 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

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5. Taylor-Robinson D, Lamont RF. Mycoplasmas in pregnancy. BJOG: An International Journal of Obstetrics & Gynaecology. 2011 Jan;118(2):164-74.

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: 506105998UPUHFW UDI-DI: (01)05061059981578 UDI-PI: See label
	Minimum and maximum storage temperatures for this product	-15 to -25 degrees Celsius
	Catalogue number	KD654208M-100
	Number of tests/reactions in this pack	100
	CE-IVD certified	CE-IVD certified