# MYCOPLASMA CONTROL

*Ureaplasma urealyticum* and *Mycoplasma hominis* quality control strains
12 tests (REF 00900)

**CPB0390_EN-2022-05**
For *in vitro* diagnostic use only, for professional use only

CE

## 1 - INTENDED USE

The MYCOPLASMA CONTROL kit contains *Ureaplasma urealyticum* (U.u) and *Mycoplasma hominis* (M.h) strains and has been designed for the quality control of liquid media methods of *in vitro* urogenital mycoplasma diagnosis (§8).

## 2 - INTRODUCTION

The quality control of analytical methods is important in the maintenance of good clinical laboratory practices. Quality control in a microbiological laboratory requires the use of known strains. These microorganisms are frequently lyophilized in order to prolong shelf-life. The detection of urogenital mycoplasmas in clinical specimens is a routine laboratory test. The main species isolated from the urogenital tract are *Ureaplasma urealyticum* (Uu) and *Mycoplasma hominis* (M.h). These bacteria are present as commensal organisms, but can be pathogenic in nature.

## 3 - PRINCIPLE

Mycoplasmas are fragile germs lacking a cell wall and are demanding in their requirement for growth factors. The lyophilized U.u and M.h strains are produced from an enriched mycoplasma broth that contains excipients to protect the microorganisms during the lyophilization process.

## 4 - REAGENTS

**MYCOPLASMA U.u**: 6 vials containing lyophilized *U. urealyticum* strain.
**MYCOPLASMA M.h:** 6 vials containing lyophilized *M. hominis* strain.
Each vial contains the lyophilized culture medium (mycoplasma broth base, foal serum, mannitol and antibiotics). The U.u and M.h strains have been isolated from clinical specimens and characterized according to standard methods (§10).

## 5 - PRECAUTIONS

The reagents in this kit are intended solely for *in vitro* use and must be handled by authorized personnel.
The reagents are potentially infectious and must be handled with caution, in observance of hygiene rules and the current regulations for this type of product in the country of use.
The MYCOPLASMA M.h. vials contain group 2 bacteria (*M. hominis*) and must be handled with caution. **Risk of contamination by a biological agent.**
Do not use strains after the expiry date.
Do not use strains that have been damaged or that have been poorly conserved before use.
After opening the kit, only remove from the refrigerator the reagents required. The U.u. and M.h. **strains are sensitive to heat and to variations in temperature.**

## 6 - PREPARATION AND STORAGE OF REAGENTS

The strains stored at 2 to 8 °C in their original packaging, are stable until the expiry date shown on the kit.
The vial of U.u. and M.h. strains should be reconstituted before use (§8)
The regenerated strains should be used immediately or stored for 24 hours at 2-8 °C maximum. Do not freeze.

## 7 - MATERIAL REQUIRED BUT NOT PROVIDED

- Sterile pipettes - Sterile distilled water
- Waste container for contaminated waste

## 8 - METHOD

Allow the reagents to reach room temperature (18 to 25 °C) before use.
Reconstitute the contents of the U.u or M.h vial with 1 mL of sterile distilled water (bacterial suspension)

### 8.1. MYCOFAST *Evolution* 2 / MYCOFAST US

Regenerate an UMMlyo (+ 2 mL UMMt) medium with an UMMt (2 mL) medium.
Inoculate this regenerated medium with a defined quantity of bacterial suspension (U.u or M.h) using the dilution mentioned on the MYCOPLASMA CONTROL kit label.
Inoculate the MYCOFAST *Evolution* 2 tray (or MYCOFAST US) as follows:
100 µL of inoculated UMM medium in each of the 10 wells (or 7 wells for MYCOFASTUS)
- 50 µL of S.Mh in wells 9 and 10 (or 6 and 7 for MYCOFAST US).
- 2 drops of mineral oil in each of the 10 wells (or 7 wells for MYCOFAST US).
Incubate the tray at 37 °C +/- 1 °C for 24 hours and interpret by comparison with the expected results:

| | $10^3$ | $10^4$ | $\geq 10^5$ | DOX | ROX | OFX | L | SXT | E | $>10^4$ |
|---|---|---|---|---|---|---|---|---|---|---|
| U.u Strain | + | + | -/+ | - | - | -/+ | + | + | - | - |
| M.h Strain | - | - | - | - | + | - | - | + | + | + |

For MYCOFASTScreening xp method follow the instructions described in § 8.1, by inoculating the MYCOFAST *EvolutioN* 2 tray after conservation of the inoculated medium during 24 hours at 2-8 °C.

### 8.2. MYCOFAST *EvolutioN* 3

Regenerate an UMMlyo (+ 3 mL UMMt) medium with an UMMt (3 mL) medium.
Inoculate this regenerated medium with a defined quantity of bacterial suspension (U.u or M.h) using the dilution mentioned on the MYCOPLASMA CONTROL kit label.
Inoculate the MYCOFAST *Evolution* 3 tray as follows:
- 100 µL of inoculated UMM medium in each of the 20 wells
- 50 µL of S.Mh in wells 6 and 7
- 2 drops of mineral oil in each of the 20 wells
Incubate the tray at 37 °C +/- 1 °C for 24 hours and interpret by comparison with the expected results:

| | $10^3$ | $10^4$ | $>10^5$ | L | SXT | E | $>10^4$ |
|---|---|---|---|---|---|---|---|
| U.u Strain | + | + | -/+ | + | + | - | - |
| M.h Strain | - | - | - | - | + | + | + |

| | DOX | PT | ROX | AZM | JM | CIP | OFX |
|---|---|---|---|---|---|---|---|
| U.u Strain | S | S | S | S | S/I | R | I/R |
| M.h Strain | S | S | R | R | S | S | S |

For MYCOFAST Screening *EvolutioN* 3 method follow the instructions described in § 8.2, by inoculating the MYCOFAST *EvolutioN* 3 tray after conservation of the inoculated medium during 24 hours at 2-8 °C.

### 8.3. MYCOFAST *RevolutioN*

Inoculate an UMMt (3 mL) medium with a defined quantity of bacterial suspension (U.u or M.h) using the dilution mentioned on the MYCOPLASMA CONTROLkit label.
Inoculate the MYCOFAST *RevolutioN* tray as follows:
- 100 µL of inoculated UMMt medium in each of the 20 wells
- 50 µL of S.Mh in wells 6 and 7
- 2 drops of mineral oil in each of the 20 wells
Incubate the tray at 37 °C +/- 1 °C for 24 hours and interpret by comparison with the expected results:

| | $10^3$ | $10^4$ | $>10^5$ | L | SXT | E | $>10^4$ |
|---|---|---|---|---|---|---|---|
| U.u Strain | + | + | -/+ | + | + | - | - |
| M.h Strain | - | - | - | - | + | + | + |

| | LVX | MXF | E | CM | TE |
|---|---|---|---|---|---|
| Strain U.u | S/R | S | S | + | S/R |
| Strain M.h | S | S | + | S | S |

### 8.4. MYCOFAST *RevolutioN* 2

Inoculate an UMMt medium (3 mL) with a quantity of bacterial suspension (U.u or M.h) determined according to the dilution indicated on the MYCOPLASMA CONTROL kit label.
Inoculate the MYCOFAST *RevolutioN* 2 gallery:
- 100 µL of UMMt medium inoculated into the 24 wells
- 2 drops of mineral oil in the 24 wells
Incubate the tray at 37 ° C +/- 1 ° C for 24 hours and interpret against the expected results below:

MYCOFAST *RevolutioN* 2

| | U.u $10^3$ | U.u $10^4$ | U.u $\geq 10^5$ | M.h$\geq 10^4$ | LVX | MXF | ERY | TET | DOX | CLI |
|---|---|---|---|---|---|---|---|---|---|---|
| Strain U.u | + | + | +/- | NA | S/R | S | S | S/R | S | NA |
| Strain M.h | NA | NA | NA | + | S | S | NA | S | S | S |

### 8.5. MYCOFAST *RevolutioN* 2 AMIES

Inoculate an UMMt AMIES medium (2.6 mL) with a quantity of bacterial suspension (U.u or M.h) determined according to the dilution indicated on the MYCOPLASMA CONTROL kit label.
Inoculate the MYCOFAST *RevolutioN* 2 gallery:
- 100 µL of UMMt medium inoculated into the 24 wells
- 2 drops of mineral oil in the 20 wells Incubate the gallery at 37 ° C +/- 1°C for 24 hours and interpret according to the expected results identical to those of (§ 8.4).

### 8.6. MYCOFAST *RevolutioN* ATB+

Inoculate an UMMt medium (3 mL) with a quantity of bacterial suspension (U.u or M.h) determined according to the dilution indicated on the MYCOPLASMA CONTROL kit label.
Inoculate the MYCOFAST *RevolutioN* ATB + gallery:
- 100 µL of UMMt medium inoculated into the 24 wells
- 2 drops of mineral oil in the 24 wells
Incubate the tunnel at 37 ° C +/- 1 ° C for 24 hours and interpret by comparison with the expected results below:

MYCOFAST *RevolutioN* ATB+

| | U.u $10^3$ | U.u $10^4$ | U.u $\geq 10^4$ | LVX | MXF | TET | ERY | CLI | TEL |
|---|---|---|---|---|---|---|---|---|---|
| U.u Strain | + | + | - | S/R | S | S/R | S | R | S |
| M.h Strain | - | - | + | S | S | S | R | S | S/R |

| | ROX | PRI | JOS | OFX | MIN |
|---|---|---|---|---|---|
| U.u Strain | S | S | S | R | S |
| M.h Strain | R | S | S | S | S |

### 8.7. MYCOFAST Screening *RevolutioN:*

Inoculate an UMMt (3 mL) medium with a defined quantity of bacterial suspension (U.u or M.h) using the dilution mentioned on the MYCOPLASMA CONTROL kit label.
Inoculate the MYCOFAST Screening *RevolutioN* tray as follows:
- 100 µL of inoculated UMMt medium in each of the U.u and M.h wells
- 50 µL of S.Mh in the M.h well
- 2 drops of mineral oil in each of the U.u and M.h wells
Incubate the tray at 37 °C +/- 1 °C for 24 hours and interpret by comparison with the expected results:

| | U.u | M.h |
|---|---|---|
| U.u Strain | + | - |
| M.h Strain | - | + |

Continue the diagnosis with COMPLEMENT MYCOFAST *RevolutioN* (00062) or COMPLEMENT MYCOFAST *RevolutioN* ATB + (00073) or COMPLEMENT MYCOFAST *RevolutioN* 2 (00082) by inoculating a MYCOFAST *RevolutioN* or -*RevolutioN* ATB+ or -*RevolutioN* 2 tray as above (§ 8.3 - 8.4 - 8.5 - 8.6) with the remaining inoculated medium, stored for 24 hours at 2-8 ° C during screening.

### 8.8. MYCOFAST Screening PLUS and MYCOFAST Screening PLUS UMMt,

Inoculate UMMt medium (3 mL) with a quantity of bacterial suspension (U.u or M.h) according to the dilution indicated on the label of the MYCOPLASMA CONTROL kit.
Proceed with the inoculation of the MYCOFAST Screening PLUS tray:
- 100 µL of UMMt medium inoculated into wells U.u and M.h
- 2 drops of mineral oil in the U.u and M.h wells

Incubate the tray at 37°C +/- 1°C for 24 hours and interpret against the expected results below:

| | U.u $\geq 10^4$ | M.h $\geq 10^4$ |
|---|---|---|
| U.u strain | + | - |
| M.h strain | - | + |

**8.9. MYCOFAST Screening PLUS UMMt FRIENDS**

Inoculate UMMt AMIES medium (2.6 mL) with a quantity of bacterial suspension (U.u or M.h) according to the dilution indicated on the label of the MYCOPLASMA CONTROL kit. Proceed with the inoculation of the MYCOFAST Screening PLUS tray:

- 100 µL of UMMt medium inoculated into wells U.u and M.h
- 2 drops of mineral oil in the U.u and M.h wells

Incubate the strip at 37°C +/- 1°C for 24 hours and interpret against the expected results below:

| | U.u $\geq 10^4$ | M.h $\geq 10^4$ |
|---|---|---|
| U.u strain | + | - |
| M.h strain | - | + |

**9 - LIMITS / CAUSES OF ERROR**

- In case of non-constant or < 36 °C incubation temperature (frequent opening of the incubator, heterogeneousness of the temperature in the incubator) the incubation can be continue for a further 2 to 4 hours.
- Poor conservation of the strains during shipment or storage.
- Non respect of the protocol.
- Use of out-of-date trays or media.

**10 - CARACTERISTICS - PERFORMANCE**

**10-1 Identification / Purity:** The U.u and M.h were identified on A7 agar plates and in U9 medium for U.u and Hayflick medium for M.h. There is no cross-contamination between U.u and M.h strains. Moreover there is no bacterial or mycological contamination on Columbia or Sabouraud agar plates.

**10-2 Enumeration:** The U.u and M.h have been enumerated in a liquid medium by using the reference method (U9 medium for U.u and Shepard medium for M.h). The MYCOFAST actual results obtained in the different trays agree with the expected results.

**10-3 Antibacterial susceptibility testing:** The antibacterial susceptibility testing results obtained with the U.u and M.h in the different trays agree with the minimal inhibitory concentrations (MIC) in liquid media.

**10-4 Repeatability and Reproducibility:** Studies carried out with two different U.u and M.h batches, for the control of MYCOFAST *Evolution* 3, demonstrated 100% repeatability and 100 % reproducibility within and between the batches.
Studies carried out to test 10 different batches of MYCOFAST *Evolution* 2 tray, MYCOFAST *Evolution* 3 tray, UMMlyo (2 mL) and UMMlyo (3 mL) media, demonstrated that there is homogeneity in the results with U.u and M.h strains.

**11 - WASTE ELIMINATION**

Waste should be disposed of in accordance with the hygiene rules and the current regulations for this kind of product in the country of use.

**12 - BIBLIOGRAPHY**

**Document Cofrac Lab GTA06. Révision 00 - Juillet 2005.** Les contrôles de la qualité analytique en biologie médicale. § 10.3.1. Contrôle de Qualité en Bactériologie.

**Ridgway, G. L., Bébéar, C., Bébéar, C. M. et al. 2001**. Antimicrobial susceptibility testing of intracellular and cell-associated pathogens. EUCAST Discussion Document E.
Dis 6.1.

**Waites, K.B., C.M. Bébear, J.A. Robertson, D.F. Talkington, and G.E. Kenny. 2001.** Cumitech 34, Laboratory Diagnosis of mycoplasmal infections. Coordinating ed., F.S.
Nolte. American Society for Microbiology, Washington, D.C.

The changes from the previous version are highlighted in grey.

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