

Urogenital mycoplasma diagnosis
MYCOFAST® Screening Revolution
Screening and differentiation

50 tests (REF 00063)

COMPLEMENT MYCOFAST® Revolution
Enumeration, identification and susceptibility testing

25 tests (REF 00062)

UMMt Revolution

50 tests (REF 00061)

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For *in vitro* diagnostic use only, for professional use only
 The changes from the previous version are underlined in grey.



1 - INTENDED USE

MYCOFAST Screening *Revolution* (REF 00063) has been designed for the screening and differentiation of *Ureaplasma urealyticum* / *Ureaplasma parvum* (U.u.) and *Mycoplasma hominis* (M.h.) in various clinical specimens. This kit should be used in association with the media contained in the UMMt *Revolution* kit (REF 00061).

In the case of positive screening, analysis can be completed with trays contained in the COMPLEMENT MYCOFAST *Revolution* kit (REF 00062) allowing the enumeration and identification of U.u and/or M.h as well as the antimicrobial susceptibility testing according to the recommendations of the CLSI (Clinical and Laboratory Standards Institute) (2).

2 - INTRODUCTION

Mycoplasmas that include several species that have been identified in humans, all belong to the mollicutes class. They differ from other bacteria in their lack of a cell wall and hence a natural resistance to β -lactams, as well as by the presence of a membrane rich in sterol obtained through their adhesion to eukaryotic cells. Since mycoplasmas are relatively fragile, they will only grow in acellular culture in the presence of various growth factors and at an optimal temperature of 37°C (4).

Most human mycoplasmas are commensal. *U. urealyticum* and *M. hominis* are the most commonly encountered species that have been isolated from the urogenital tract. *U. urealyticum* species are divided into two biovars: *U. urealyticum* and *U. parvum* (U.u.).

U.u. and M.h. can be pathogenic. They are responsible for male genital infections (non-gonococcal urethritis, epididymitis, prostatitis, infertility); female genital infections (bacterial vaginosis, endometritis, salpingitis); fertility problems (chorioamnionitis, post-partum endometritis, preterm birth, spontaneous abortion), neonatal problems (low birth weight, respiratory and neurological infections, bacteremias, abscesses); extragenital infections (septic arthritis, reactive arthritis, other infection loci) (1).

The diagnosis of mycoplasma infections depends upon the determination of the pathological threshold, followed by enumeration. The resistance of U.u./M.h. to certain drugs necessitates antimicrobial susceptibility testing (5, 6). The drugs tested and the interpretation criteria are adapted for the treatment of infections caused by mycoplasmas encountered in the urogenital tract or in extragenital sites (2).

3 - PRINCIPLE

MYCOFAST Screening *Revolution* is a liquid method based on the ability of U.u. and M.h. to metabolize urea and arginine respectively. The mycoplasma growth results in a colour change of the medium, containing phenol red indicator, from yellow-orange to red. This colour change is due to liberation of ammonia resulting in an alkaline pH of the medium.

Mycoplasma growth thus viewed enables:

- detection and differentiation; then, if a positive result is obtained:
- the enumeration of mycoplasma based on the rate of urea or arginine hydrolysis, which is proportional to the number of germs contained in the sample.
- the identification based on the sensitivity or otherwise of the germ to three antimicrobial agents.
- the U.u and M.h susceptibility testing to antimicrobial agents.

4 - REAGENTS

Description	Amount		
	#00061	#00062	#00063
UMMt : Vial of 3 mL mycoplasma broth with antimicrobial agents and preservative solution. pH : 6.0 ± 0.1	50	-	-
MYCOFAST SCREENING <i>Revolution</i> : Divisible tray of 10 wells for 5 tests, individually packed in an aluminium sachet with an integrated desiccant	-	-	10
Labels: Sheet of 5 divisible labels	-	-	10
S. Mh.: <i>Mycoplasma hominis</i> growth activator (4.5 mL)	-	2	1
MYCOFAST <i>Revolution</i> : Tray of 20 wells for 1 test, packed in an aluminium sachet with an integrated desiccant	-	25	-
Closing system: Protective translucent plastic lid for MYCOFAST <i>Revolution</i> tray	-	25	-

MYCOFAST Screening Revolution tray

Tray consisting of 5 rows of 2 wells: a *Ureaplasma urealyticum* (U.u) well containing lincomycin and urea and a *Mycoplasma hominis* (M.h) well containing erythromycin and arginine.

MYCOFAST Revolution tray

The MYCOFAST *Revolution* tray contains in each of the 20 wells the dehydrated culture medium (foal serum, yeast extract, cysteine, arginine, urea, phenol red, antibiotics, pH: 6.1 ± 0.1) and comprises 4 parts:

- Wells 1-3 Enumeration for U.u. between 10³ and ≥10⁵ CCU/mL (buffered solution and lincomycin included to inhibit the growth of M.h).
- Wells 4-6 U.u. and M.h. Identification via resistance profiles to Lincomycin (L), Trimethoprim/Sulfamethoxazole (SXT) and Erythromycin (E).
- Well 7 Enumeration of M.h.: ≥ 10⁴ CCU/mL (buffered solution and erythromycin included to inhibit the growth of U.u).
- Wells 8-20 Antimicrobial susceptibility testing for U.u. and M.h. against: Levofloxacin (LVX) 1-2-4 µg/mL, Moxifloxacin (MXF) 0.25-2 µg/mL, Erythromycin (E) 8-16 µg/mL, Clindamycin (CM) 0.25-0.5 µg/mL, Tetracycline (TE) 1-2-4-8 µg/mL.

5 - PRECAUTIONS

- The reagents are intended solely for *in vitro* use and must be handled by authorized personnel.
- The patient samples and inoculated reagents are potentially infectious; they must be handled with caution, in observance of hygiene rules and the current regulations for this type of product in the country of use.
- Reagents containing raw materials of animal origin must be handled with caution.
- Do not use reagents after the expiry date.
- Do not use reagents that have been damaged or that have been poorly conserved before use.
- A positive result with the MYCOFAST method indicates colonization by urogenital mycoplasmas, but cannot alone be used to make a clinical diagnosis. This must be made by a doctor and is a function of the biological results and clinical signs.

6 - SAMPLE COLLECTION AND HANDLING

6.1 Sample collection

Cervicovaginal sample collection : Use only a Dacron or rayon swab or a cytobrush to collect samples. The cervix should be carefully cleaned with a swab, to remove secretions, before collecting the sample with a new swab. As mycoplasmas adhere strongly to mucous cells, the mucous lining should be vigorously scrubbed to obtain a rich specimen.

Urethral sample collection : Clean the meatus and swab or scrape the area to obtain cells.

Sperm, Urine : Collect sperm or first micturition in a sterile tube or bottle.

Gastric secretions : Collect gastric secretions from the neonate by aspiration with a catheter and transfer to a sterile bottle.

6.2 Transport in UMMt medium

Swab samples: Place the swab in a vial of UMMt medium.

Liquid samples: Inoculate a vial of UMMt medium with 300 µL of homogenized liquid.

6.3 Conservation in UMMt medium

The inoculated UMMt medium may be kept for 20 hours at room temperature (18-25°C) or 56 hours at 2-8°C.

For storage during 3 days at -20°C, first add 2 drops of "MYCOPLASMA Stabilizer".

7 - PREPARATION AND STORAGE OF REAGENTS

- All the reagents are ready-to-use. The vials may be stored at 2-8 °C, in their original packaging until the expiry date shown on the kit.

- Should only one or two, three, or four rows of (U.u) (M.h) wells be used, the remaining MYCOFAST Screening *Revolution* tray may be stored for 4 weeks at 2-8 °C in its original packaging and hermetically resealed.

- The UMMt medium may be stored temporarily at room temperature but is more stable at 2 - 8°C.

- The S.Mh supplement is stable for 3 months after opening

- Do not freeze the reagents contained in the kit.

8 - MATERIAL REQUIRED BUT NOT PROVIDED

- Sample collection (Swabs, cytobrushes, sterile containers for liquid samples), pipettes and tips
- MYCOPLASMA Stabilizer (REF 00064); Incubator at 37°C ± 1°C
- Waste container for contaminated waste and mineral oil

9 - METHOD

Allow the reagents to reach room temperature (20-30 minutes).

9.1 SCREENING - MYCOFAST Screening Revolution tray

- Prepare as many rows of wells as samples to be tested.
- If required separate one or several rows of (U.u)/(M.h) wells with the aid of the marks found on the tray.

9.1.1 Inoculation of UMMt Revolution medium

Seed the UMMt medium with a swab or 300 µL of liquid sample (§6.2). Mix well.

9.1.2 Inoculation of Uu/Mh wells

- Distribute successively:
- (U.u) well: 100 µL of seeded UMMt medium.
- (M.h) well: 100 µL of seeded UMMt medium.
50 µL of Mh supplement.

- Add 2 drops of mineral oil to the two wells.
- Cover the wells with the divisible labels and label the sample in order to identify it.

- **Store excess seeded UMMt medium at 2-8°C** in order to continue the analysis in case of positive screening.

9.1.3 Incubation of Uu/Mh wells

Incubate the wells of the tray for 24 hours at 37°C ± 1°C. Tray incubation can be extended for up to 48 hours only in the case of liquid samples that are negative after 24 hours.

9.1.4 Reading and interpretation of Uu/Mh wells

- Check that the 2 (U.u) (M.h) wells are limpid. A cloudy appearance in a well indicates bacterial contamination. In this case repeat the analysis. Observe the colour change of the medium in the U.u and M.h wells: U.u wells are orangey or red: Presence of *Ureaplasma urealyticum* M.h wells are orangey or red: Presence of *Mycoplasma hominis* U.u / M.h wells are yellow: Absence of mycoplasma

In the case of positive screening continue diagnosis with the MYCOFAST Revolution tray.

9.2 ENUMERATION, IDENTIFICATION AND SUSCEPTIBILITY TESTING

9.2.1 Inoculation of the MYCOFAST Revolution tray

- Remove the adhesive film by pulling on the tab and add the following to the wells of each row:

- Wells 1-20 100 µL of inoculated UMMt
- Wells 6-7 50 µL of S.Mh supplement
- Wells 1-20 2 drops of mineral oil

- Cover the seeded tray with the "closing system".

● Label the sample.
Store excess UMMt medium at 2-8°C for at least 48 hours for possible verification.

9.2.2 Incubation of the tray

Incubate the tray at 37°C ± 1°C for 24 hours. For U.u. and M.h enumeration, read the results in 24 hours. Tray incubation can be extended for up to 48 hours only in the case of liquid samples that are negative after 24 hours.

9.2.3 – Reading and interpretation

Check that all the wells in the row are limpid. A cloudy appearance in a well indicates bacterial contamination. In this case repeat the analysis. The results are read by the colour obtained in the different wells. Urogenital Mycoplasma growth is indicated when the medium turns red (alkaline). The medium remains yellow when no growth of urogenital mycoplasma occurs. An orangey coloration should be considered as a positive test (rate limit).

For the interpretation of the results refer to the results sheet.

