# MYCOFAST® US

**Urogenital Mycoplasma Diagnosis**

- Detection
- Enumeration
- Identification

**30 tests (**Cat N° 00050)

EN-2007-03

CE

## 1 - INTENDED USE

MYCOFAST US has been designed for the detection, enumeration and identification of *Ureaplasma urealyticum* (U.u.) and *Mycoplasma hominis* (M.h.) in endocervical, urethral, urinary, gastric and sperm specimens.

## 2 - INTRODUCTION

Mycoplasmas are the smallest and simplest of the procaryotypes capable of self-reproducing (0.15 to 0.25 µm) (1). 15 human species have been found to date all belonging to the mollicutae class (derived from: *cutis mollis*; soft skin). They differ from other bacteria in their lack of a cell wall and hence a natural resistance to ß-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 cultures in the presence of various growth factors and at a constant temperature of 35 to 37 °C.

Most human mycoplasmas are commensal. Of the 9 species that have been isolated from the urogenital tract, *Ureaplasma urealyticum* and *Mycoplasma hominis* are the most commonly found. U.u. and M.h. are sexually transmitted and can be pathogenic. Respiratory infections or meningitis can occur in the neonate as a result of contamination from the genital tract at birth (3). In adults, the infections caused by U.u. and M.h. are described in the table below (6) :

| | *U. urealyticum* | *M. hominis* |
|---|---|---|
| Non-gonoccocal urethritis | ++++ | - |
| Urethro-prostatitis | ++ | - |
| Epididymitis | +++ | - |
| Urinary stones | ++ | - |
| Pyelonephritis | - | ++++ |
| Pelvic inflammation | - | +++ |
| Post partum fever/abortion | ++ | ++++ |
| Chorioamnionitis | ++ | - |

Conventional diagnosis is based upon culture on A7 agar plates followed by microscopicidentification of U.u. (sea urchin shaped) or M.h. (fried-egg shaped) colonies.

Since both U.u. and M.h. are commensal, infection can only be diagnosed through the determinination of the pathological threshold, followed by precise enumeration.

## 3 - PRINCIPLE

MYCOFAST US identifies U.u. and M.h. growth after a 24 hour incubation in a liquid medium. During growth, U.u. and M.h. metabolize urea and arginine respectively resulting in a color change of the medium, which contains phenol red indicator, from yellow-orange to red. This color change is due to liberation of ammonia resulting in an alkaline pH of the medium.

Mycoplasma growth thus viewed enables:

The enumeration of mycoplasmas based on the rate of urea or arginine hydrolysis, which is proportional to the number of organisms contained in the sample. (European patent # 0311541 ; US Patent # 5.091.307).

The identification based on the resistance profile to three antibiotics at chosen concentrations:

- *U. urealyticum* in liquid medium is resistant to Lincomycin, whereas *M. hominis* is susceptible.
- *M. hominis* in liquid medium is resistant to Erythromycin, whereas *U. urealyticum* is susceptible.
- *U. urealyticum* and *M. hominis* are resistant to antifolates of the Trimethoprim / Sulfamethoxazole.

## 4 - REAGENTS

| Description | Amount |
|---|---|
| **UMMt**: Vial of mycoplasma transport medium (2 mL) | **30** |
| **UMMlyo**: Vial of lyophilized growth medium | **30** |
| **MYCOFAST US Tray:** Tray of 2 x 10 wells sealed in an aluminium package. | **15** |
| **S. M.h.** : *Mycoplama hominis* growth activator (4.5 mL) | **2** |

***UMMt medium - Composition in g/L of distilled water:***

Mycoplasma broth.....20
Antibiotics.....10 mL
pH : 6.0 ± 0.2

***Reconstituted medium (UMMt+UMMlyo) - Composition in g/l of distilled water:***

Mycoplasma broth.....20
Foal serum.....200 mL
Yeast extract.....5.8
Cysteine.....0.3
Arginine.....9
Urea.....3.6
Phenol red.....0.04
Antibiotics.....10 mL
pH : 6.1 ± 0.1

***MYCOFAST US tray***

Each tray consists of 2 tests, each with 10 wells. Each test has 3 parts:

| Wells | Purpose |
|---|---|
| **1-3** | *Enumeration for U.u.* between $10^3$ and $\geq 10^5$ CCU/mL |
| **4-6** | *U.u. and M.h. identification* via resistance profiles to Lincomycin (L), Trimethoprim /Sulfamethoxazole (SXT) and Erythromycin (E) |
| **7** | *Enumeration of M.h.* ($\geq 10^4$ CCU/mL |
| **8-10** | Empty wells |

Specific U.u. enumeration is carried out by including lincomycin in the first 3 wells, which inhibit M.h. growth, (if present). To enumerate M.h. in well 10, erythromycin is included to inhibit the growth of U.u.

## 5 - PRECAUTIONS

- The reagents are intended solely for *in vitro* use and must be handled by authorized persons.
- The patient samples and inoculated reagents are potentially infectious; they must be handled with caution, observing universal precautions and the current regulations for this type of product in the country of use.
- Handle reagents containing raw materials of animal origin with caution.
- Do not use reagents after the expiration date.
- Do not use damaged or poorly stored reagents
- A positive result with the MYCOFAST method indicates colonisation by urogenital mycoplasmas, but cannot alone be used to make a clinical diagnosis. Clinical diagnosis 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

***Endocervical and vaginal 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 well scraped to obtain a rich specimen** (2).

***Urethral sample collection***

Clean the meatus and swab or scrape the area to obtain cells.

***Sperm, Urine***

Collect sperm or first micturition (urine) in a sterile tube or bottle.

***Gastric secretions***

Collect gastric secretions from neonates by aspirating through a catheter and transferring 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 200 µL of homogenized liquid.

**The inoculated UMMt medium may be kept for 8 hours at room temperature (18-25 °C) or 16 hours at 2-8 °C.**

## 7 - PREPARATION AND STORAGE OF REAGENTS

- All the reagents are ready to use. Store the vials at 2-8 °C, in their original packaging until the expiration date shown on the kit.
- The UMMt medium may be stored temporarily at room temperature but is more stable at 2-8 °C.
- Should only half a tray be used, the remaining half may be stored, prior to use, for up to 3 weeks at 2-8 °C in its original packaging.
- The 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 collecting material (swabs, cytobrushes, sterile containers for liquid samples)
- Pipettes and tips
- Waste container for contaminated waste
- Paraffin oil
- Incubator at 37 °C ± 1 °C

## 9 - METHOD

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

### 9.1 Regeneration of UMMlyo medium

Transfer each inoculated UMMt medium into a new UMMlyo vial. The UMMlyo medium thus regenerated should display an orange tint. Mix well.

### 9.2 Inoculation of the tray

- Identify each series of wells.
- Remove the adhesive film and add the following to the wells of each row:

| | |
|---|---|
| Wells 1-7 | 100 µL of inoculated UMMlyo |
| Wells 6-7 | 50 µL of S.Mh supplement |
| Wells 1-7 | 2 drops of paraffin oil |

- Recover the wells with the adhesive film.

**Store excess UMMlyo medium in its vial** at 2-8 °C for at least 48 hours for possible verification.

### 9.3 Incubation of the tray

Incubate tray at 37 °C ± 1 °C for 24 hours.
For U.u. and M.h enumeration, read the results in 24 hours. Prolong the incubation for up to 48 hours to enable strains with a weak enzymatic activity to become apparent.
Note: Incubation can be continued for up to 72 hours for urine, sperm and gastric secretions if necessary.

## 10 - READING AND INTERPRETATION

### 10.1 Validation

Check that all the wells in the row are clear. A cloudy appearing well indicates bacterial contamination. In this case repeat the analysis.

### 10.2 Reading and interpretation

The results are read by the color 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.
Note: An orange tint should be considered as a positive test (rate limited).

### 10.2.1 Identification (wells 4, 5 and 6)

Identification is made according to the color change of specific wells, as well as the observations in wells 4, 5 and 6 which determine the profile :

| | Color in well # | | |
|---|---|---|---|
| | **4 (L)** | **5 (SXT)** | **6 (E)** |
| ***Ureaplama urealyticum*** | red | red | yellow |
| ***Mycoplasma hominis*** | yellow | red | red |

### 10.2.2 Enumeration (wells 1, 2, 3 and 7)

Mark the wells that have turned red and interpret:

| **Red color observed in well #** | **Interpretation (CCU/mL)** |
|---|---|
| 1 | U.u. value $10^3$ |
| 1 and 2 | U.u. value $10^4$ |
| 1, 2 and 3 | U.u. value $\geq 10^5$ |
| 7 | M.h. value $\geq 10^4$ |

***Notes***

- For males, the interpretation criterion for *U. urealyticum* is $\geq 10^4$ CCU/mL for an urethral sample; for females the interpretation criterion for *M.hominis* is $\geq 10^4$ CCU/mL for an endocervical sample (5).
- The concentration usually quoted for *U. urealyticum* in a first urine stream, sperm, or an endotracheal specimen is $10^3$ CCU/mL (5).

## 11 - PARTICULAR CASES

**Very high U.u and M.h levels**

The content of all the wells on the tray has turned red. It is recommended that the sample be diluted in order to obtain more specific results. In this case, proceed as follows:

- Inoculate a new UMMt vial with 200 µL of the original UMMlyo medium stored at 2-8 °C (see § 9.2). Ensure that no color change has taken place on removing the medium from the refrigerator.
- Regenerate a new UMMlyo vial with the whole inoculated UMMt vial.
- Inoculate a new tray with the medium obtained (§ 9.3)
- Take the dilution (1:10) into account in the interpretation of the enumeration results.
- If necessary, confirm the presence of mycoplasmas on an A7 agar plate by reisolating from the original UMMlyo medium stored at 2-8 °C (see § 9.2).

## 12 - QUALITY CONTROL

Quality control can be carried out from a lyophilized reference strain *(Ureaplasma urealyticum* ATCC 27618).
Prepare a preculture after inoculation of 100µL of the regenerated strain in a vial of reconstituted UMM medium (UMMt+UMMlyo).
Incubate at 37 °C ± 1 °C for 6 hours.
Inoculate a new UMM broth reconstituted with 200 µL of the homogenized preculture.
Inoculate the MYCOFAST US tray and perform the test as indicated in these instructions (§9.2, 9.3, and 10).

***Expected result:***

| $10^3$ | $10^4$ | $\geq 10^5$ | L | SXT | E | $\geq 10^4$ |
|---|---|---|---|---|---|---|
| + | + | - | + | + | - | - |

Note: In the event of a lyophilized strain with low ureasic activity, incubation can be continued for up to 18-24 hours. The broth should be inoculated with 20 µL of preculture.

## 13 - LIMITATIONS OF THE PROCEDURE

- Some bacteria that are present in quantities of $>10^{6-7}$ CFU/mL and contain urease may cause all the wells in the tray to change color. The presence of these can be verified by reisolating on chocolate agar from the original UMMlyo medium stored at 2-8 °C (see § 9.2).
- A basic sample pH (pH > 8) may lead the UMM medium to change color. Should this occur, dilute the sample (1:10) in fresh UMM medium and interpret the results taking the dilution into account.
- As with all organism search methods, the quality of the sample can influence the test result. A negative test does not necessarily indicate the absence of infection.
- A sample with a low mycoplasma load ($<10^3$ CCU/mL) may lead to a random color change in the different wells in the tray.

## 14 - PERFORMANCE

Clinical samples were used to test the performance of the MYCOFAST method by comparison with culture on A7 agar plates. The results of the identification are summarized in the following table:

| | **SAMPLES** | | |
|---|---|---|---|
| | **Urethral Vaginal** | **Sperm** | **Urine** |
| **n=** | 100 | 150 | 150 |
| **Prevalence** | 52% | 13.3% | 16% |
| **Sensitivity** | 98.2% | 88% | 86.4% |
| **Specificity** | 100% | 98.4% | 98.4% |

The MYCOFAST method has also been validated for the detection and identification of mycoplasmas in samples of neonate gastric secretions (n=208, prevalence 19.2%) and in parallel on cervico-vaginal samples from the mother (n=208, prevalence 48.1%). All the strains of mycoplasma present on the A7 agar were detected with the MYCOFAST method (4).

## 15 - WASTE ELIMINATION

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

## 16 - BIBLIOGRAPHY


**1. BEBEAR C., DE BARBEYRAC B.** 1994. Les mycoplasmes, p. 1443-1463. Dans FRENEY J., RENAUD F. , HANSEN W. , BOLLET C. (éd.).“Manuel de bactériologie clinique”, 2ème éd., vol.3, Elsevier, Paris .

**2. BOUCAUD-MAITRE Y. et THOINET S.** 1993. Analyse des prélèvements en bactériologie médicale - 2ème partie : prélèvements génitaux. Feuil. Biol., **34** : 21-24.

**3. GENIAUX M.** 1994. Microbiologie des MST chez les mineurs de moins de 15 ans. Méd Mal Infect. **24**:409-424.

**4. GRATTARD F., SOLEIHAC B., DE BARBEYRAC B., BEBEAR C., SEFFERT P. and POZZETTO B.** 1995. Epidemiologic and molecular investigations of genital mycoplasmas from women and neonates at delivery. Pediatr. Infect. Dis. J. , **14** : 853-858.

**5. PEREYRE S., BEBEAR C.M., BEBEAR C.** 2001. Les mycoplasmes en pathologie humaine. Revue Française des Laboratoires. Supplément au N°**329**, 34-36.

**6. TAYLOR-ROBINSON D.** 1995. *Ureaplasma urealyticum* (T-strain Mycoplasma) and *Mycoplasma hominis*, p. 1713-1718. Dans MANDELL G. L. , BENNET J. E. and DOLIN R. (ed.). Principles and Practices of Infectious Diseases, 4th ed., vol. 2, Churchill Livingstone, New York.





**Place of manufacture**
ELITech France
Parc d’activités
Allée d’Athènes
83870 SIGNES
(FRANCE)

**USA DISTRIBUTOR**
WESCOR, INC
**459 South Main Street**
**Logan, Utah** 84321
Tel: 435 752 6011
Fax: 435 752 4127

1

1