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 mollicutes class (derived from: cutis molis; soft skin). 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 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 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.

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 antifoamites of the Trimethoprim / Sulfamethoxazole.

4 - REAGENTS

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>UMMt: Vial of mycoplasma transport medium (2 mL)</td>
<td>30</td>
</tr>
<tr>
<td>UMMt: Vial of lyophilized growth medium</td>
<td>30</td>
</tr>
<tr>
<td>MYCOFAST US Tray: Tray of 2 x 10 wells sealed in an aluminium package</td>
<td>15</td>
</tr>
<tr>
<td>S. Mh.: Mycoplasma hominis growth activator (4.5 mL)</td>
<td>2</td>
</tr>
</tbody>
</table>

UMMt medium - Composition in g/L of distilled water:
- Mycoplasma broth (20 g/L)...
- Antibiotics (10 mL)
- pH : 6.0 ± 0.2

Reconstituted medium (UMMt+UMMlyo) - Composition in g/L of distilled water:
- Mycoplasma broth (20 g/L)...
- Foal serum (200 mL)
- Yeast extract (5.8 g/L)
- Cysteine (0.3 g/L)
- Arginine (0.9 g/L)
- Urea (3.6 g/L)
- Phenol red (0.04 g/L)
- 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 10³ and ≥10⁵ CCU/mL
  - 4-6 U.u. and M.h. identification via resistance profiles to Lincomycin (L), Trimethoprim (T) and Sulfamethoxazole (SXT) and Erythromycin (E)
  - 7 Enumeration of M.h. (≥ 10⁴ 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.

Pelvic inflammation - ++++
Pyelonephritis - ++++
Urinary stones ++ -
Epididymitis+++ -
Urethro-prostatitis ++ -
Non-gonoccocal urethritis  ++++ -

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

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

9.2 Incubation 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 UMMyo
  - 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 UMMyo 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. urealyticum and M. hominis enumeration, read the results in 24 hours. Prolong the incubation 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:

10.2.2 Enumeration (wells 1, 2, 3 and 7)
Mark the wells that have turned red and interpret:

<table>
<thead>
<tr>
<th>Red color observed in well #</th>
<th>Interpretation (CCU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>U. u. value (10^4)</td>
</tr>
<tr>
<td>1 and 2</td>
<td>U. u. value (10^4)</td>
</tr>
<tr>
<td>1, 2 and 3</td>
<td>U. u. value (10^5)</td>
</tr>
<tr>
<td>7</td>
<td>M. h. value (10^4)</td>
</tr>
</tbody>
</table>

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 \(\geq 10^5\) CCU/mL for an endocervical sample.
- The concentration usually quoted for \(U. urealyticum\) in a first urine stream, sperm, or an endo-tracheal specimen is \(10^5\) CCU/mL.

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:

- Incubate a new UMMyo vial with 200 µL of the original UMMyo 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 UMMyo vial with the whole inoculated UMMt vial.
- Incubate 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 UMMyo medium stored at 2-8 °C (see §9.2).

12 - QUALITY CONTROL

Quality control can be carried out from a lyophilized reference strain \((Ureaplastma urealyticum\ ATCC 27618)\). Prepare a pre-culture after inoculation of 100 µL of the regenerated strain in a vial of reconstituted UMM medium (UMMt+UMMyo).

Incubate at 37 °C ± 1 °C for 6 hours.

Inoculate a new UMMt broth reconstituted with 200 µL of the homogenized pre-culture.

Inoculate the MYCOFAST US tray and perform the test as indicated in these instructions (§9.2, 9.3, and 10).

Expected result:

\[10^3 < 10^4 \quad > 10^5 \quad \text{L} \quad \text{SXT} \quad \text{E} \quad > 10^4\]

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

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 homogenizing on chocolate agar from the original UMMyo 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:

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>Urethral</th>
<th>Vaginal</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence</td>
<td>100%</td>
<td>52%</td>
<td>88%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>98.2%</td>
<td>13.3%</td>
<td>86.4%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
<td>98.4%</td>
<td>98.4%</td>
</tr>
</tbody>
</table>

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


MYCOFAST® is a trademark of ELITech France SAS

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