MYCOSCREEN *PLUS*

Detection and differentiation of urogenital mycoplasmas

16 tests (Ref. 00024)
32 tests (Ref. 00025)

US-2007-09

CE

1 - INTENDED USE

MYCOSCREEN *PLUS* has been designed for the detection and differentiation of *Ureaplasma urealyticum* (U.u.) and *Mycoplasma hominis* (M.h.) in clinical specimens. MYCOSCREEN *PLUS* must be used in association with the A7 Agar plate for the enumeration and the microscopical identification of U.u. and M.h. Each kit contains 16 or 32 tests.

2 - INTRODUCTION

Mycoplasmas are the smallest and simplest of the prokaryotes 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 encountered. 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	++	-

MYCOSCREEN *PLUS* is a method that enables the differential diagnosis between commensal germs ($\leq 10^3$CCU*/ml) and germs that could be incriminated in an infectious or super infectious process ($\geq 10^4$CCU/ml). *Colour Change Unit.
MYCOSCREEN *PLUS* must be associated with a conventional diagnosis based upon culture on A7 Agar plates, (modified Shepard medium) (5).

3 - PRINCIPLE

MYCOSCREEN *PLUS* is a 24-hour method based upon the ability of U.u. and M.h. to metabolise, respectively, urea and arginine. Mycoplasma growth in a liquid medium is visualised by the colour change of an indicator from yellow to fuchsia pink. This is due to the alkalinization of the medium as a result of the release of ammonia.
MYCOSCREEN *PLUS* is carried out in a divisible microplate enabling the testing of 4 samples. Each microplate is composed of 4 rows of 2 wells: a *Ureaplasma urealyticum* (U.u) well containing lincomycin and a *Mycoplasma hominis* (M.h) well containing erythromycin.

4 - REAGENTS

Description	Quantity	
	#00024	#00025
MYCOSCREEN *PLUS* tray: Divisible microplate of 4 rows of 2 wells (U.u) / (M.h),	4	8
U.M.M.t : Vial of mycoplasma transport medium (2 ml)	16	32
U.M.M.lyo : Vial of lyophilised growth medium (+2ml UMMt)	16	32
S. M.h. : *Mycoplama hominis* growth activator (4.5ml)	1	1

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

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 MYCOSCREEN *PLUS* method indicates colonisation 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

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 in a sterile tube or bottle.

Gastric secretions
Collect gastric secretions from the neonate 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 200µl of homogenised 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. The vials may be stored at 2-8°C, in their original packaging until the expiry date shown on the kit.
- When a single row of wells (U.u.) (M.h.) or two or three rows of wells are used, the remaining wells on the MYCOSCREEN *PLUS* tray can be conserved in the original aluminium packet, sealed hermetically, for up to 3 weeks at 2-8°C.
- The Mh supplement is stable for 3 months after opening.

8 - MATERIAL REQUIRED BUT NOT PROVIDED

- Sample collecting material (Swabs, cytobrushes, sterile containers for liquid samples)
- Pipettes and tips (100µl)
- Waste container for contaminated waste
- Paraffin oil
- Incubator at 37°C ± 1°C
- A7 AGAR (#00079) or (#00090)

9 - METHOD

9.1 Preparation of reagents

Allow the reagents to reach room temperature
- Prepare the same number of rows of wells, as there are samples to be tested.
- If necessary, cut out one or several rows of wells (U.u)(M.h) with the aid of the notches.
- Label each test.

9.2 Inoculation of UMM medium

- Seed the UMMt medium with the swab or 200 µl of liquid sample. (§6.2).
- Mix well
- Transfer all of the UMMt medium to the UMMlyo vial. Do not dispose of the empty UMMt vial.
- Mix well

9.3 Inoculation of the tray wells

- Distribute successively:
  (U.u) well: 100 µl of inoculated UMMlyo
  (M.h) well: 100 µl of inoculated UMMlyo
  50 µl of Mh supplement.
- Add two drops of paraffin oil to each of the wells.
- Remove 500 µl of seeded UMMlyo medium and transfer it to the empty UMMt vial.
- Store excess inoculated UMMlyo in its vial at 2-8°C, so that in the case of a positive screening/ detection result, an A7 Agar culture can be carried out (§ chapitre11).

Note: Certain *M. hominis* strains exhibit a weak enzymatic activity. The addition of a drop of Mh Supplement allows a colour change to be visualized within 24 hours for strains whose concentration is $\geq 10^4$CCU/ml.

9.4 Incubation

Incubate tray wells and the UMMt vial at 37°C ± 1°C for 24 hours, then read the results.
For enumeration, read the tray results within 24 hours.
Prolong the incubation for up to 48 hours for the rates inferior to 10^4CCU/ml or to enable strains with a weak enzymatic activity to become apparent.

10 - READING AND INTERPRETATION

10.1 Validation

Check that the 2 wells (U.u) (M.h) are limpid. A cloudy appearance indicates bacterial contamination.
In this case, repeat the analysis.

10.2 Reading and interpretation

Urogenital Mycoplasma growth in the wells is indicated by the alkalinization of the medium, which changes colour from yellow to red.
The medium remains yellow when no growth of urogenital Mycoplasma occurs.
An orangey colouring should be considered as a positive test.

10.2.1 Reading within 24 hours

Red colour change in the U.u well:
Strong probability that *Ureaplasma urealyticum* at a supra-pathological rate is present in the sample ($\geq 10^4$CCU/ml).
Red colour change in the M.h well:
Strong probability that *Mycoplasma hominis* at a supra-pathological rate is present in the sample ($\geq 10^4$CCU/ml).
Red colour change in the U.u and M.h wells:
Strong probability that *Ureaplasma urealyticum* and *Mycoplasma hominis* at supra-pathological rates are present in the sample ($\geq 10^4$CCU/ml).
Note: Very strong concentrations of Uu or Mh can cause the medium in the two wells to change color.
Yellow colour in the U.u and M.h wells and red colour in the UMMt vial
Presence of mycoplasma at a rate of $<10^4$CCU/ml.
In this case, seed an A7 Agar plate (§12) especially for samples other than the endocervical or urethral in origin (§13).
Yellow colour in the U.u and M.h wells and yellow colour in the UMMt vial
Absence of mycoplasmas, or presence of mycoplasmas at a rate of $<10^3$CCU/ml.
Reincubate the tray and the vial.

10.2.2 Reading within 48 hours

Yellow colour in the U.u and M.h wells and red colour in the UMMt vial
Probablity that mycoplasmas at a rate of $<10^2$CCU/ml are present in the sample.
Yellow colour in the U.u and M.h wells and red colour in the UMMt vial
Absence of mycoplasmas.

11 - QUALITY CONTROL

Quality control can be carried out from a lyophilised 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 200µl of the homogenised preculture.
Inoculate the two wells of the MYCOSCREEN *PLUS* tray and perform the test as indicated in these instructions (§9 et 10).

Expected results:	U.u	M.h
	+	-

Note: In the event of a lyophilised 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.

12 - CULTURE ON A7 AGAR PLATE

If the screening is positive, take the seeded UMMlyo medium conserved at 2-8°C and inoculate an A7 Agar plate with 100 µl of the homogenised suspension.
Incubate at 37°C ± 1°C in an anaerobic environment for 48 hours.

12.1 Morphological identification

***Ureaplasma urealyticum*:** Appearance of a brown-black precipitate (variable size, "sea urchin shaped"). The colonies are small.
Mycoplasma hominis: "fried egg shaped" appearance. The colonies are larger than those of *U. urealyticum*.

12.2 Enumeration

Enumeration is performed upon an upside down agar plate with the aid of an optical microscope (x10 objective). The number of colonies per field of view is recorded:
- Less than 1 colony / field: $\leq 10^3$ CFU/ml
- 1 to 5 colonies / field: approx. 10^4 CFU /ml
- 5 to 10 colonies / field: approx. 10^5 CFU /ml
- 10 to 20 colonies / field: approx. 10^6 CFU /ml
- > 20 colonies / field: > 10^6 CFU /ml

CFU: Colony Forming Units

13 – SPECIFIC CASES

For sperm, urine, surgical salpingitis specimens, haemoculture, quality control of cell culture, etc, it is important to determine the concentration of mycoplasmas present. It is preferable that an A7 Agar plate is seeded with an inoculated UMMlyo medium, without waiting for the screening results.
The concentration usually quoted for *U. urealyticum* in a first urine stream, sperm, or an endotracheal specimen is 10^3 CCU/ml (4).

14 - LIMITATIONS OF THE PROCEDURE

- The presence of *Ureaplasma urealyticum* or *Mycoplasma hominis* at very high concentrations can cause the medium in the two wells to change color.
- Some bacteria that are present in quantities of $>10^{6\text{-}7}$ CFU/ml and contain urease may cause the two wells to change colour.
- A basic sample pH (pH > 8) may lead the UMM medium to change colour.
- As for all germ search methods, the quality of the sample can influence the test result. A negative test does not therefore necessarily indicate the absence of infection.

15 - PERFORMANCE

A study carried out on 334 clinical specimens (224 endocervical specimens, 25 urethral specimens, 75 sperm specimens, 5 urine specimens and 5 miscellaneous specimens), of which 99 specimens were positive, gave the following results compared with the MYCOFAST *Evolution* 2 method, for rates of 10^4 CCU/ml.

Detection - 24 hours	***U. urealyticum***	***M. hominis***
Sensitivity	92.7%	100%
Specificity	98.8%	99.3%
PPV	95.5%	91.6%
NPV	98.1%	100%

Detection - 48 hours	***U. urealyticum***	***M. hominis***
Sensitivity	98.8%	95.7%
Specificity	98.7%	98.2%
PPV	96.6%	90%
NPV	99.5%	99.2%

With the MYCOSCREEN *PLUS* tray 75,2% of the *U.urealyticum* strains and 48,2% of the *M. hominis* strains were detected after 24 hours of incubation. For a detection threshold of 10^4CCU/ml the results were similar to those obtained with the MYCOFAST *Evolution* 2 tray.

Differentiation of U.u and M.h
The global concordance for the differentiation within 24 hours between *U. urealyticum* and *M. hominis,* compared to the MYCOFAST *Evolution* 2 method is 96.5%.

16 - 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.

17 - BIBLIOGRAPHY

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