Urogenital mycoplasma diagnosis

# MYCOFAST<sup>®</sup> Screening *RevolutioN*

Screening and differentiation

50 tests (REF 00063)

# COMPLEMENT MYCOFAST<sup>®</sup> RevolutioN

Enumeration, identification and susceptibility testing

# 25 tests (REF 00062) UMMt RevolutioN

50 tests (REF 00061)

# CPB 0396 EN-2015-12

For in vitro diagnostic use only, for professionnal use only The changes from the previous version are underlined in grev.

# 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 ß-lactams, as well as by the presence of a membrane rich in sterol obtained through their adhesion to eukarvotic 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. urealvticum and M. hominis are the most commonly encountered species that have been isolated from the urogenital tract. U. urealvticum species are divided into two biovars: U. urealvticum and U. parvum (U.u.).

U.u. and M.h. can be pathogenic. They are responsible for male genital infections (non-gonoccocal urethritis, epididymitis, prostatitis, infertility); female genital infections (bacterial vaginosis, endometritis, salpingitis); fertility problems (chorioamniotitis, post-partum endometritis, preterm birth, spontaneous abortion), neonatal problems (low birth weight, respiratory and neurological infections, bacteremias, abcesses); 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 - <u>REAGENTS</u>			
Description		Amount	
	#00061	#00062	#00063
<b>UMMt</b> : Vial of 3 mL mycoplasma broth with antimicrobial agents and preservative solution. pH : 6.0 ± 0.1	50	-	-
<b>MYCOFAST SCREENING</b> <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.: Mycoplama hominis growth activator (4.5 mL)	-	2	1
MYCOFAST RevolutioN : 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 RevolutioN tray	-	25	-

# **MYCOFAST Screening RevolutioN tray**

Tray consisting of 5 rows of 2 wells: a Ureaplasma urealyticum (U.u) well containing lincomvcin 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 10<sup>3</sup> and >10<sup>5</sup> 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). Enumeration of M.h.:  $\geq$  10<sup>4</sup> CCU/mL (buffered solution and
- Well 7 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 vigourously scrabbeb 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 transferral 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 trav

- 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
- 50 uL of S.Mh supplement Wells 6-7
- 2 drops of mineral oil Wells 1-20
- 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 trav

Incubate the trav at  $37^{\circ}C \pm 1^{\circ}C$  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.

DEAGENTS

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

Identification is n	nade according	g to the colour change in v	vells 4, 5 and 6:					
	4 (L)	5 (SXT)	6 (E)					
U. urealyticum	red	red	yellow					
M. hominis	yellow	red	red					
Enumeration (we	ells 1, 2, 3 and	7)						
Mark the wells that have turned red and interpret:								
1	U.u. value	10 <sup>o</sup> CCU/mL						
1 and 2	U.u. value	10 <sup>4</sup> CCU/mL						
1 2 and 2		<100 CCU/ml						

1, 2 and 3	0.u. value > 10% CCU/mL
7	M.h. value >10 <sup>4</sup> CCU/ml

The pathological role of mycoplasmas in urogenital infections is subject to interpretation according to specific recommendations (1,3,7).

The pathological thresholds usually quoted for U. urealyticum are: >104 CCU/mL for a urethral specimen or endotracheal specimen, >103 CCU/mL in a first urine stream or sperm (although a new local recommendation mentions a threshold > 104 CCU / ml for semen (7)). The presence of M. hominis at a threshold > 104 CCU/mL in a cervicovaginal specimen is abnormal (1, 3).

Antimicrobial susceptibility testing (wells 8 to 20) The red colour change of the medium in the wells containing an antibiotic indicates the presence of bacterial growth and hence resistance to the antibiotic concentration being tested. The yellow colour of the medium indicates the absence of bacterial growth and hence susceptibility to the antibiotic concentration being tested. The strains are characterized as being sensitive or resistant to the antibiotics according to the following criteria defined by the CLSI: MIC (ug/mL) Interpretative Criteria

Antimicrobial		U.u		M.h		Comments
Class	Drug	S	S R S R		R	
Quinolones	Levofloxacin	<u>≤</u> 2	<u>≥</u> 4	<u>≤</u> 1	<u>≥</u> 2	
Quinoiones	Moxifloxacin	≤2		<u>≤</u> 0.25		
Macrolides	Erythromycin	<u>≤</u> 8	<u>&gt;</u> 16			Organisms susceptibles to erythromycin will also be susceptible to azythromycin
Lincosamides	Clindamycin			<u>≤</u> 0.25	<u>&gt;</u> 0.5	
Tetracyclines	Tetracycline	<u>&lt;</u> 1	<u>≥</u> 2	<u>≤</u> 4	<u>≥</u> 8	Organisms susceptibles to tetracycline will also be susceptible to doxycycline

• The strain is said to be **Sensitive** when its growth is inhibited by the higher and lower critical concentrations of the antibiotic.

 The strain is said to be Resistant when its growth is inhibited by the higher critical concentration of the antibiotic, but not the lower critical concentration or when its growth is not inhibited by either the higher or the lower critical concentrations of the antibiotic.

For Moxifloxacine only one concentration is tested for U.u. and M.h.

 M. hominis strains are innately resistant to macrolides (14 -15 carbon atoms). including erythromycin.

 In some patient populations, tetracycline resistance is as high as 45% for U.u and 39.6% for M.h (2). U.u/M.h guinolone (5, 6) and clindamycin resistance have been described but the prevalence is not known.

**10 - PARTICULAR CASES** For 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 300 µL of the original UMMt medium stored at 2-8°C (see § 9.2.1).

Inoculate a new tray with the new inoculated UMMt medium.

•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 re-isolating from the original UMMt medium stored at 2-8°C (§ 9.2.1).

• A non-constant incubation temperature or <36°C (frequent opening and temperature heterogeneity of the incubator) can slow down the mycoplasmas arowth kinetics.

#### 11 - QUALITY CONTROL

Quality control can be carried out from a MYCOPLASMA CONTROL (REF 00900) strain or from a lyophilised reference strain (*Ureaplasma urealyticum* ATCC 33175) previously calibrated at 10<sup>4-5</sup> UCC/mL.

<u>Screening</u>: Inoculate the two wells of the MYCOFAST Screening *Revolution* tray and perform the test as indicated in these instructions (§ 9.1). Expected results: U.u (+) and M.h (-).

Identification, enumeration and susceptibility testing: Inoculate the MYCOFAST *Revolution* tray and perform the test as indicated in these instructions (§ 9.2).

Expected results (ATCC 33175):

Uu	Uu	Uu	L	SXT	E	Mh	LVX	LVX	LVX	
+	+	+/-	+	+	-	-	+/-	-	-	
MXF	MXF	E	E	CM	CM	TE	TE	TE	TE	
+/-	-	-	-	+	+	+	+	+	+/-	

# **12 - LIMITATIONS OF THE PROCEDURE**

**12.1 - Screening** The MYCOFAST Screening *RevolutioN* tray allows detection at a threshold of <10<sup>3</sup> UCC/mL and does not enable numeration. The numeration obtained with the MYCOFAST Revolution trav can appear negative following positive screening.

 Some bacteria that are present in quantities of ≥10<sup>6-7</sup> CFU/ml and contain grease may cause all the wells in the tray to change colour. The presence of these can be verified by re-isolating on chocolate agar from the original UMMt medium stored at 2-8°C (see § 9.2).

• An alkaline sample  $pH(pH \ge 8)$  may lead the UMM medium to change colour. Should this occur, dilute the sample (1:10) in fresh UMM medium and interpret the results taking the dilution into account.

A sample with a low mycoplasma load (< $10^3$  CCU/mL) may lead to a random colour change in the different wells of the tray.

• As for all germ detection methods, the quality of the sample can influence the test result. A negative test does not therefore necessarily indicate the absence of infection.

#### **13 - PERFORMANCE** 13.1 Isolated strains

#### 13.1.1 Screening and differentiation - Identification - Enumeration

A comparative study was carried out with 9 isolated strains (ATCC strains and collection strains) tested separately (U.u or M.h) with two concentrations or mixed together (U.u/M.h). The results obtained were compared with those obtained with another method in liquid medium.

MYCOFAST Screening RevolutioN tray

- For the screening (n=19) there was 100% concordance.

- For the differentiation (n=21), all of the strains U.u or M.h tested were correctly identified in the wells of the MYCOFAST Screening RevolutioN tray.

# MYCOFAST RevolutioN tray

- For the identification (n=21) there was 100% concordance.

- For the numeration of U.u (N = 11) 10 tests were concordant and 1 test gave a numeration with 10<sup>3</sup> UCC/mL with MYCOFAST RevolutioN and >10<sup>4</sup> UCC/mL with the comparative method (≤ 10<sup>3</sup> UCC/mL with A7 AGAR).

- For the numeration of M.h (N = 10) 6 tests were concordant and 4 tests gave a numeration >10<sup>4</sup> UCC/mL with MYCOFAST RevolutioN and <10<sup>4</sup> UCC/mL with the comparative method (10<sup>4</sup> UCC/mL with A7 AGAR).

# 13.1.2. Antimicrobial susceptibility testing

A comparative study was carried out in a national reference laboratory between the determination of minimal inhibitory concentration (MIC) in liquid medium and the MYCOFAST RevolutioN method. The strains tested (5 U. urealyticum, 10 U. parvum et 10 M. hominis) were reference collection strains, clinical wild-type strains or strains having acquired resistance. Each strain was tested at dilutions  $10^3$ ,  $10^4$  and  $10^5$  CCU/mL. The results of the two methods were interpreted as being Susceptible (S) or Resistant (R) according to the CLSI M43-A standard recommendations.

	U.urealyticum/parvum (n = 45)					<i>M. hominis</i> (n = 30)			
	LVX MXF E TE				LVX	MXF	CM	TE	
Concordance	43	42	45	44	30	30	29	30	
ME	2	3	0	0	0	0	0	0	
VME	0	0	0	1*	0	0	1**	0	

For *U. urealvticum* the overall agreement is 96.7 % (174/180).

For *M. hominis* the overall agreement is 99.2% (119/120). Concordance : (S/S ou R/R), ME : Major Error (R/S), VME : Very Major Error (S/R) \*: Discrepancy obtained at 10<sup>4</sup> UCC/mL within one dilution (MIC at 2 µg/mL) \*\*: Discrepancy obtained at 10<sup>5</sup> UCC/mL within one dilution (MIC at 0.5 µg/mL)

13.2 <u>Clinical strains</u> A comparative study was carried out with clinical samples collected in double, of which 179 positive samples (U.u and/or M.h) detected by at least one of the two methods. The results obtained with MYCOFAST *RevolutioN* were compared with those obtained with the routine method used in test laboratories. 13.2.1 Identification

Positive samples for U.u: The U.u strains detected with the comparative method were correctly identified with MYCOFAST RevolutioN except for 2 samples.

5 samples that were negative with the comparative method appeared positive for U.u with MYCOFAST RevolutioN.

Positive samples for M.h: The 4 M.h strains detected with the comparative method were correctly identified with MYCOFAST RevolutioN.

Positive samples for U.u and M.h: The samples that were positive for U.u and M.h with the comparative method appeared positive for U.u and M.h with MYCOFAST RevolutioN. except for 5 samples. 11 positive samples for U.u. and 2 negative samples with the comparative method appeared positive for U.u and M.h with MYCOFAST RevolutioN.

	n = 179	MYCOFAST RevolutioN	COMPARATIVE METHOD
	111	U.u	U.u
U.u (n = 118)	5	U.u	Absence
	2	Absence	U.u
M.h (n = 4)	4	M.h	M.h
	39	U.u/M.h	U.u/M.h
	11	U.u/M.h	U.u
U.u/M.h (n = 57)	2	U.u/M.h	Absence
	4	U.u	U.u/M.h
	1	M.h	U.u/M.h

### 13.2.2. Enumeration

The enumeration results of the U.u (n = 175) and M.h strains (n = 61) obtained with the comparative method and/or MYCOFAST RevolutioN are described in the following two tables.

		-						
U.u (n=175)	MYCOFAST RevolutioN	COMPARATIVE METHOD		M.h	MYCOFAST RevolutioN	COMPARATIVE METHOD		
147	≥ 10 <sup>5</sup> CCU/mL	≥ 10 <sup>4</sup> CCU/mL		(n=61)	Revolution	METHOD		
2	$\geq 10^5 \text{ CCU/mL}$	< 10 <sup>4</sup> CCU/mL	]	10	≥ 10 <sup>4</sup> CCU/mL	$\geq 10^4$ CCU/mL		
5	<u>&gt;</u> 10 <sup>5</sup> CCU/mL	Absence		34				
9	10 <sup>4</sup> CCU/mL	$\geq 10^4$ CCU/mL	1	- 34	≥ 10 <sup>-+</sup> CCU/mL	< 10 <sup>4</sup> CCU/mL		
1	10 <sup>4</sup> CCU/mL	Absence		13	≥ 10 <sup>4</sup> CCU/mL	Absence		
5	10 <sup>3</sup> CCU/mL	$\geq 10^4$ CCU/mL	1			4		
1	10 <sup>3</sup> CCU/mL	< 10 <sup>4</sup> CCU/mL		4	Absence	< 10 <sup>4</sup> CCU/mL		
1	10 <sup>3</sup> CCU/mL	Absence	/	Among the 165 positive samples f				
2	Absence	≥ 10 <sup>4</sup> CCU/mL		Among the 165 positive samples fo U.u with the two methods, 150 samples show an identica				
1	Absence	< 10 <sup>4</sup> CCU/mL	e	enumerat	tion. Amona t	an identical the 44 positive		
	2	4	complex for M builth the two methods					

< 10<sup>3</sup> CCU/mL < 10<sup>4</sup> CCU/mL samples for M.h with the two methods, 10 samples show an identical

enumeration and 34 samples show an enumeration with a pathological threshold with MYCOFAST *RevolutioN* method and an infra-pathological threshold with the comparative method.

# 14 - 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.

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6 - WAITES KEN B, DONNA M. CRABB, and LYNN B. DUFFY. 2008. Comparative In Vitro Activities of the Investigational Fluoroquinolone DC-159a and Other Antimicrobial Agents against Human Mycoplasmas and Ureaplasmas. ANTIMICROBIAL AGENTS AND CHEMOTHERAPY Vol 52 No 10 3776-3778

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