MYCOFAST® *RevolutioN 2*

Urogenital Mycoplasma Diagnosis

Detection
Enumeration
Identification
Antimicrobial susceptibility testing
25 tests (REF 00080)

CPB 0410_EN-2023-08
For *in vitro* diagnostic use only, for professional use only

CE

I – INTENDED USE

MYCOFAST *RevolutioN 2* has been designed for the detection, enumeration and the identification of *Ureaplasma Urealyticum* / *Ureaplasma parvum* (U.u.) and *Mycoplasma hominis* (M.h.) in various clinical specimens. In addition, MYCOFAST *RevolutioN 2* can be used to determine the susceptibility of U.u. and M.h. to certain antimicrobial agents 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 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-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, respi- ratory 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 adap- ted for the treatment of infections caused by mycoplasmas encountered in the urogenital tract or in extragenital sites (2).

3 – PRINCIPLE

MYCOFAST *RevolutioN 2* is a liquid method based on the ability of U.u. and M.h. to metabolize urea and arginine respectively. Mycoplasma growth results in a colour change of the medium, containing phenol redindicator, from yellow-orange to red. This colour change is due to libera-tion of ammonia resulting in an alkaline pH of the medium. Mycoplasma growth thus viewed enables:

- 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 U.u. and M.h. susceptibility testing to antimicrobial agents.

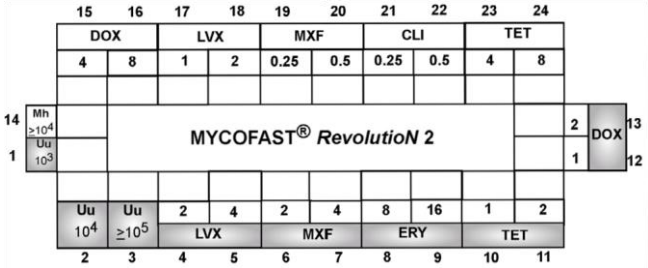
In the case of mixed samples (U.u. + M.h.), the test allows the interpretation of the sensitivities of each species with regard to the antibiotics tested.

4 – REAGENTS

Description	Amount
UMMt: Vial of 3 mL mycoplasma broth with antimicrobial agents and preservative solution. pH: 6.0 ± 0.1	25
MYCOFAST® *RevolutioN 2*: Tray of 24 wells packed in an aluminium sachet with an integrated desiccant.	25
Closing system: Protective translucent plastic tray lid.	25

The MYCOFAST *RevolutioN 2* tray, in each of the 24 wells, contains dehydrated mycoplasma culture medium (foal serum, yeast extract, cysteine, arginine, urea, phenol red, antibiotics, pH : 6.1 ± 0.1) and includes 2 separate parts :

- the part intended for enumeration and antibiotic susceptibility testing of the U.u. species (wells identified on the label as black)
- the part intended for enumeration and antibiotic susceptibility testing of the M.h. species (wells identified on the label as red)



Diagnosis of U.u. species (black part of tray label):

Wells 1/2/3 : Identification and enumeration of U.u. at 10^3,10^4 et ≥10^5 CCU/mL (buffered solution lincomycin inhibiting M.h. growth).
Wells 4/5 : Evaluation of U.u. susceptibility to Levofloxacin (LVX) at 2 / 4 µg/mL
Wells 6/7 : Evaluation of U.u. susceptibility to Mocifloxacin (MXF) at 2 / 4 µg/mL
Wells 8/9 : Evaluation of U.u. susceptibility to Erythromycin (ERY) at 8 / 16 µg/mL
Wells 10/11 : Evaluation of U.u. susceptibility to Tetracycline (TET) at 1 / 2 µg/mL
Wells 12/13 : Evaluation of U.u. susceptibility to Doxycycline (DOX) at 1 / 2 µg/mL

Wells 4 to 13 contain Urea (specific substrate of the U.u. species) and Lincomycin (inhibitor of the growth of M.h.).

Diagnosis of M.h. species (red part of tray label):

Wells 14 : Identification and enumeration of M.h. at ≥10^4 CCU/mL (buffered solution and Erythromycin inhibiting the growth of U.u.)
Wells 15/16 : Evaluation of M.h. susceptibility to Doxycycline (DOX) at 4 / 8 µg/mL
Wells 17/18 : Evaluation of M.h. susceptibility to Lévofloxacin (LVX) at 1 / 2 µg/mL
Wells 19/20 : Evaluation of M.h. susceptibility to Moxifloxacin (MXF) at 0.25 / 0.5 µg/mL
Wells 21/22 : Evaluation of M.h. susceptibility to Clindamycin (CLI) at 0.25 / 0.5 µg/mL
Wells 23/24 : Evaluation of M.h. susceptibility to Tétracycline (TET) at 4 / 8 µg/mL

Wells 15 to 24 contain Arginine (specific substrate of the M.h. species) and Erythromycin (inhibitor of the growth of U.u.).

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 according to 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 collectinf the sample with a new swab. As mycoplasmas adhere strongly to mucous cells, the mucous lining should be vigorously swabbed 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.

6.2 Transport in UMMt medium

Swab samples : Place the swab in a vial of UMMt 3 mL medium.
Liquid samples : Inoculate a vial of UMMt 3 mL 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.
The UMMt medium may be stored temporarily (3 months) at room temperature but is more stable at 2-8 °C.

8 – MATERIAL REQUIRED BUT NOT PROVIDED

Sample collection (swabs, cytobrushes, sterile containers for liquid samples), pipettes and tips
MYCOPLASMA Stabilizer (REF 00064) if storage of the sample in the UMMt for 3 days at -20°C; 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 Inoculation of the tray

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

Wells 1-24	100 µL of inoculated UMMt medium
Wells 1-24	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 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 within 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.

10 – READING AND INTERPRETATION

10.1 Validation

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.

10.2 Reading and interpretation

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).
In the case of a result read in 48h (for a liquid sample with a negative test result in 24h), only interpret the presence of the detected mycoplasma without enumeration result.
For the interpretation of the results refer to the results sheet.

10.2.1 Enumeration (wells 1, 2, 3 et 14)

The wells that have turned red are identified and interpreted as follows:

1	U.u. 10^3 CCU/mL
1 and 2	U.u. 10^4 CCU/mL
1, 2 and 3	U.u. ≥ 10^5 CCU/mL
14	M.h. ≥ 10^4 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:
≥10^4 CCU/mL for a urethral specimen or endotracheal specimen, >10^3 CCU/mL in a first urine stream or sperm (although a new local recommendation mentions a threshold ≥10^4 CCU/mL for semen (7). The presence of *M. hominis* at a threshold ≥10^4CCU/mL in an cervicovaginal specimen is abnormal (1, 3).

10.2.2 Susceptibility tests (wells 4 to 13 and 15 to 24)

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 (2):

Table of MIC (µg/mL) interpretative criteria

Antibiotic		U.u.		M.h.		Comments
Class	Drug	S	R	S	R	
Quinolones	Levofloxacin	≤2	≥4	≤1	≥2	
	Moxifloxacin	≤2	≥4	≤0.25	≥0.5	
Lincosamides	Clindamycin			≤0.25	≥0.5	
Tétracydines	Tetracydine	≤1	≥2	≤4	≥8	
	Doxycycline	≤1	≥2	≤4	≥8	
Macrolides	Erythromycin	≤8	≥16			Organisms susceptibles to Erythromycin will also be susceptible to Azithromycine

Help with interpretation:

Susceptibility testing for U.u.

Antibiotic	LVX			MXF			ERY			TET			DOX		
Concentration (µg/mL)	2	4	int*	2	4	int*	8	16	int*	1	2	int*	1	2	int*
Profile	-	-	S	-	-	S	-	-	S	-	-	S	-	-	S
	+	-	R	+	-	R	+	-	R	+	-	R	+	-	R
	+	+	R	+	+	R	+	+	R	+	+	R	+	+	R

int*= interpretation

Susceptibility testing for M.h.

Antibiotic	LVX			MXF			CLI			TET			DOX		
Concentration (µg/mL)	1	2	int*	0.25	0.5	int*	0.25	0.5	int*	4	8	int*	4	8	int*
Profile	-	-	S	-	-	S	-	-	S	-	-	S	-	-	S
	+	-	R	+	-	R	+	-	R	+	-	R	+	-	R
	+	+	R	+	+	R	+	+	R	+	+	R	+	+	R

int*= interpretation

The strain is said to be susceptible when its growh 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.
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. quinolone (5, 6) and clindamycin resistance have been described but the prevalence is not known.

11 – 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 3 mL vial with 300 µL of the original UMMt medium stored at 2-8°C (see § 9.1).
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 plateby re-isolating from the original UMMt medium stored at 2-8°C (§ 9.1). A non-constant incubation temperature or <36°C (frequent opening andpoor temperature heterogeneity of the incubator) can slow down the mycoplasma growth kinetics.

12 – QUALITY CONTROL

Quality control can be carried out from the lyophilized *U. urealyticum* or *M. hominis* strains of the MYCOPLASMA CONTROL kit (REF 00900) or from lyophilized reference strains (*U. urealyticum* ATCC 27815 or *M. hominis* ATCC 23114) previously calibrated at $10^{4\text{-}5}$ UCC/mL.
Inoculate the MYCOFAST *RevolutioN 2* tray and perform the test as indicated in these instructions (§9 et 10)
Expected results (ATCC):

MYCOFAST *RevolutioN* 2

	U.u. 10^3	U.u. 10^4	U.u. ≥10^5	M.h. ≥10^4	LVX	MXF	ERY	TET	DOX	CLI
Strain U.u. ATCC 27815	+	+	+/-	-	S	S	S	S/R	S	NI*
Souche M.h. ATCC 23114.	-	-	-	+	S/R	S	NI*	S	S	S

NI* (Not Interpretable)

13 – LIMITES OF THE PROCEDURE

Some bacteria that are present in quantities of >$10^{6\text{-}7}$CFU/mL and contain urease 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 (§ 9.1).
A sample with an alkaline pH (pH ≥8) may cause the UMM medium to change colour. Should this occur, dilute the sample (1:10) in another UMMt medium and interpret the results taking the dilution into account.
A sample with an acidic pH (pH ≤5) can slow down the appearance of the colour change.
A sample containing blood may cause a colour change in the wells of the MYCOFAST *RevolutioN* 2 tray and could be interpreted as a positive result. In this case dilute the sample (1:10) in another UMMt medium and interpret the results, taking into account the dilution.
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.

14 – PERFORMANCE

14.1 Identification – Enumeration

% of overall agreement	U.u.	M.h.	U.u./M.h.
isolated strains (threshold ≤ 10^3 CCU/mL) (see § 14.1.1)	97.4	NA*	NA*
isolated strains (threshold ≥ 10^4 CCU/mL) (see § 14.1.1)	93.4	93.4	93.4
vaginal samples (see § 14.1.2)	100	100	100
urinary clinical specimens (see § 14.1.2)	93.2	96.6	94.9

NA* (Not Applicable)

14.1.1 Isolated strains

A comparative study was carried out with 21 isolated strains (ATCC strains and collection strains) tested separately (U.u. or M.h.) with severalconcentrations (76 tests in total).
The results obtained were compared with those obtained with the micro-dilution enumeration method.
For interpretation with a pathological threshold set at 10^3 CCU/mL, the overall agreement for U.u. is 97.4% (we listed 2 false positives at 10^2 CCU/mL with the micro-dilution enumeration method).
For interpretation with a pathological threshold of 10^4 CCU/mL; the overall agreement for U.u. is 93.4% (we recorded 5 false positives at10^3 CCU/mL with the micro dilution enumeration method). The overall agreement for M.h. is 93.4% (we listed 5 false positives, 4 at 10^3 CCU/mL and one at 10^2 CCU/mL with the micro dilution enumeration method).
The overall U.u. + M.h. agreement is 93.4%.

14.1.2 Clinical samples

An initial comparative study was performed using vaginal clinical specimens (n =23) on dry swabs. The results obtained with MYCOFAST *RevolutioN* 2 were compared with the liquid micro-dilution enumeration method. The overall agreement for U.u. and M.h. is 100%.

A second comparative study was performed on urinary clinical specimens (n=88).
The results obtained with MYCOFAST *RevolutioN* 2 were compared with those obtained with the liquid micro-dilution enumeration method.

The overall agreement for U.u. is 93.2% (we listed 1 false negative at 10^4 CCU/mL with micro-dilution numeration method and 5 false positives at 10^2 CCU/mL with with micro-dilution numeration method).
The overall agreement for M.h. is 96.6% (we identified 3 false positives at 10^2 – 10^3 CCU/mL with micro-dilution numeration method).The overall agreement for U.u. and M.h. is 94.9%.

14.2 Susceptibility testing

A comparative study was carried out in a national reference laboratory between the method for determining the minimum inhibitory concentrations (MIC) in liquid medium and the MYCOFAST *RevolutioN* 2.
The tested strains (7 *U. urealyticum*, 11 *U. parvum* and 16 *M. hominis*) were reference strains, wild-type clinical strains or strains with acquired resistance. Each strain was tested at 10^3, 10^4 and 10^5 CCU/mL dilutions in UMMt 3 mL.
For 10^4 and 10^5 CCU/mL rates, results were read and interpreted after 24 hours of incubation.
For 10^3 CCU/mL rate, results were read and interpreted after 48 hours incubation in case of negative test in 24 hours.
The results of both methods were interpreted as susceptible (S) or resistant (R) according to CLSI recommendations.
The overall agreement for *Ureaplasma urealyticum* / *Ureaplasma parvum* is: 95.5%.
The overall agreement for *Mycoplasma hominis* for rates at 10^4-10^5 CCU/mL is: 100%.

	***Ureaplasma urealyticum* / *parvum* (n=40)**					***Mycoplasma hominis* (n=28)**				
	TET	**DOX**	**MOX**	**LVX**	**ERY**	**TET**	**DOX**	**MOX**	**LVX**	**CLI**
Agreement	**34**	**38**	**40**	**39**	**40**	**28**	**28**	**28**	**28**	**28**
ME	**5[a]**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
VME	**1[b]**	**2[c]**	**0**	**1[d]**	**0**	**0**	**0**	**0**	**0**	**0**

ME: Major Error, VME : Very Major Error

[a] : 1 discrepancy at 10^3 CCU/mL (MIC of reference 0.5 µg/mL), 4 discrepancies at 10^5 CCU/mL (MIC of reference 0.5 - 1 and 8 µg/mL).
[b] : 1 discrepancy at 10^5 CCU/mL (MIC of reference 8 µg/mL).
[c] : 1 discrepancy at 10^3 CCU/mL (MIC of reference 8 µg/mL); .1 discrepancy at 10^5 CCU/mL (MIC of reference 2 µg/mL)
[d] : 1 discrepancy at 10^5 CCU/mL (MIC of reference 4 µg/mL).

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

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The changes from the previous version are highlighted in grey.

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