MYCOFAST® *RevolutioN 2* AMIES

Urogenital Mycoplasma Diagnosis

Detection

Enumeration

Identification

Antimicrobial susceptibility testing

25 tests (REF 00081)

CPB 0411_EN-2023-08

For *in vitro* diagnostic use only, for professional use only

CE

I - INTENDED USE

MYCOFAST *RevolutioN 2* AMIES has been designed for the detec- tion, enumeration and the identification of *Ureaplasma urealyticum* / *Ureaplasma parvum* (U.u.) and *Mycoplasma hominis* (M.h.) in various clinical specimens prepared with AMIES transport medium or a universal transport medium for viruses, chlamydias, mycoplasmas and ureaplas- mas. In addition, MYCOFAST *RevolutioN 2* AMIES 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 LaboratoryStandards 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 bac- teria in their lack of a cell wall and hence a natural resistance to ß-lac- tams, as well as by the presence of a membrane rich in sterol obtained through their adhesion to eukaryotic cells. Since mycoplasmas are re- latively 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, 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 *RevolutioN 2* AMIES 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 red indicator, 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.

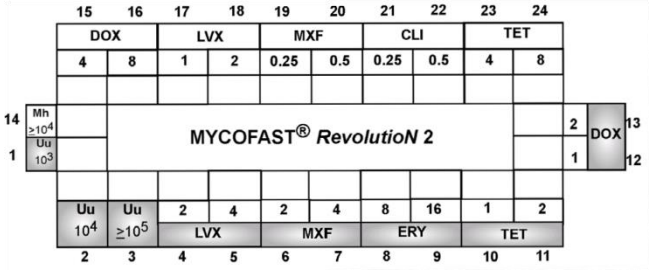
4 - REAGENTS

Description	Amount
UMMt AMIES: Vial of 2.6 mL mycoplasma broth with antimicrobial agents and preservative solution. pH: 6.0 ± 0.1	25
MYCOFAST® *RevolutioN 2:* Tray of 24 wells packed inan 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 in- cludes 2 separate parts:

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

-the part intended for enumeration and 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 and $\geq 10^5$ CCU/mL (buffered solution and 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 Moxifloxacin (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 $\geq 10^4$ 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 Levofloxacin (LVX) at 1/2 µg/mL
Wells19/20 : 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 toTetracycline (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

Only use the swab provided with the transport medium.

The cervix should be carefully cleaned with a swab, to remove secretions, before collection 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

Only use the swab provided with the transport medium.

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

6.2 Transport of samples

Transport in AMIES medium or a universal transport medium for viruses, chlamydias, mycoplasmas and ureaplasmas:

Refer to the manufacturer's operating instructions.

Transport in UMMt AMIES medium :

Place 300 µL of the inoculated transport medium in a vial of UMMt AMIES medium.

6.3 Conservation of the samples

Conservation in AMIES medium or a universal transport medium for viruses, chlamydias, mycoplasmas and ureaplasmas:

Refer to the manufacturer's operating instructions.

Conservation in UMMt AMIES medium :

The inoculated UMMt AMIES 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 AMIES 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 materials (swabs provided with the AMIES transport medium or a universal transport medium for viruses, chlamydias, my- coplasmas and ureaplasmas), 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 UMMt AMIES vial

If the sample has been transported in UMMt AMIES medium inoculated with 300 µL of AMIES medium or a universal transport medium for viruses, chlamydias, mycoplasmas and ureaplasmas; **then pass directly to step 9.2.**

If the sample has been transported in AMIES medium or a universal transport medium for viruses, chlamydias, mycoplasmas and ureaplasmas, then transfer 300 µL of this transport medium into a vial of UMMt AMIES.

9.2 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 AMIES medium
Wells 1-24	2 drops of mineral oil

Cover the seeded tray with the “closing system”.
Label the sample.
Store excess UMMt AMIES medium at 2-8°C for at least 48 hours for possible verification.

9.3 Incubation of the tray

Incubate the tray at 37°C ± 1°C for 24 hours.

Tray incubation must 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 and 14)

Mark the wells that have turned orange or red and interpret:

1	U.u. value 10^3 CCU/mL
1 and 2	U.u. value 10^4 CCU/mL
1, 2 and 3	U.u. value ≥10^5 CCU/mL
14	M.h. value ≥ 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 ≥ 10^4 CCU/mL in a cervicovaginal specimen is abnormal (1, 3).

10.2.2 Susceptibility testing (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 Susceptible 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	/
Tetracydines	Tetracydine	≤1	≥2	≤4	≥8	/
	Doxycycline	≤1	≥2	≤4	≥8	/
Macrolides	Erythromycin	≤8	≥16	/	/	Organisms susceptibles to erythromycin will also be susceptible to azythromycin

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		
Concen-tration (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 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.
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 AMIES 2.6mL vial with 260 µL of the original UMMt AMIES medium stored at 2-8°C (see § 9.2).
Inoculate a new tray with the new inoculated UMMt AMIES 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). A non-constant incubation temperature or <36°C (frequent opening and poor temperature heterogeneity of the incubator) can slow down the mycoplasma growth kinetics.

12 – QUALITY CONTROL

Quality control can be carried out with the lyophilized *U. urealyticum* or *M. hominis* strains of the MYCOPLASMA CONTROL kit (REF 00900) or from a lyophilized reference strain (*U. urealyticum* ATCC 27815 or *M. hominis* ATCC 23114) previously calibrated at $10^{4\text{-}5}$ CCU/mL.
Inoculate the MYCOFAST *RevolutioN 2* tray and perform the test as indicated in these instructions (§ 9 and 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*
Strain M.h. ATCC 23114.	-	-	-	+	S/R	S	NI*	S	S	S

NI* (Not interpretable)

13 – LIMITATIONS 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).
An alkaline sample pH (pH ≥8) may lead the medium to change colour. Should this occur, dilute the sample (1:10) in fresh UMMt AMIES 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 – PERFORMANCES

14.1 Identification – Enumération

% of overall agreement		U.u.	M.h.	U.u./M.h.
Isolated strains (threshold ≤ 10^3 CCU/mL) (see § 14.1.1 and note 1)	24h reading	88.9	NA*	NA*
	read as in § 9.3	86.7	NA*	NA*
Isolated strains (threshold ≥ 10^4 CCU/mL) (see § 14.1.1 and note 1)	24h reading	91	96.4	93.7
	read as in § 9.3	82.1	92.5	87.3
vaginal clinical samples (see § 14.1.2)	read as in § 9.3	88.2	100	94

NA* : not applicable

14.1.1 Isolated strains

A comparative study was carried out using 26 isolated strains (ATCC strains and collection strains) tested separately (U.u. or M.h.) on 3 trans- port medium references (Sigma Transwab and Sigma VCM from Medi- cal Wire and ESwab Collection Kit from BD) at several concentrations (a total of 279 tests).
The results obtained are compared with those obtained using the micro-dilution liquid method.
For interpretation with a pathological threshold set at 10^3 CCU/mL and a reading of the result at 24h, the overall agreement for U.u. is 88.9% (we listed 14 false positives: 13 at 10^2 CCU/mL and one below 10^2 CCU/mL and 17 false negatives: 11 at 10^3 CCU/mL - 5 at 10^4 CCU/mL and one at 10^5 CCU/mL enumeration method).
For interpretation with a pathological threshold set at 10^4 CCU/mL and a reading of the result at 24h; the global concordance for U.u. is 91% (we have listed 18 false positives: at 10^2 - 10^3 CCU/mL and 7 false negatives: at 10^4 – 10^5 CCU/mL with the micro-dilution enumeration method).
The overall agreement for M.h. is 96.4% for a reading of the result at 24h (we recorded one false positive at 10^3 CCU/mL, and 9 false negatives at10^4 CCU/mL with the micro-dilution enumeration method).
The global agreement U.u. + M.h. with a reading of the result at 24h is 93.7%.
For interpretation with a pathological threshold set at 10^3 CCU/mL and a reading of the result according to the protocol described in the leaflet (§ 9.3); the overall agreement for U.u. is 86.7% (35 false positives were recorded: 34 at 10^2 CCU/mL and one below 10^2 CCU/mL and 2 false negatives: at 10^3 CCU/mL with the micro-dilution enumeration method).
For interpretation with a pathological threshold set at 10^4 CCU/mL and a reading of the result according to the protocol described in the leaflet (§ 9.3); the overall agreement for U.u. is 82.1% (we listed 49 false positives: at rate at 10^2 - 10^3 CCU/mL and 1 false negative: at 10^4 CCU/mL with the micro-dilution enumeration method).
The overall agreement for M.h. is 92.5% for a reading of the result according to the protocol described in the leaflet (§ 9.3) (we have listed 21 false positives at a rate of 10^2 - 10^3 CCU/mL with the micro-dilution enumeration method)).
The global agreement U.u. + M.h. with a reading of the result according tothe protocol described in the notice (§ 9.3) is 87.3%.

14.1.2 Clinical samples

An initial comparative study was performed using vaginal clinical specimens (n =59) carried out on 3 types of transport medium and their associated swabs (Sigma Transwab and Sigma VCM from Medical Wire and ESwab Collection Kit from BD). The results obtained with MYCOFAST *RevolutioN* 2 AMIES were compared with the liquid micro-dilutionenumeration method.
The overall agreement for U.u. is 88.2% (we identified 3 false negative at 10^4 – 10^5 – 10^6 CCU/mL and 4 false positive at <10^2 – 10^2 and 10^3 CCU/mL with micro-dilution numeration method).
The overall agreement for M.h. is 100%
The overall agreement for U.u. and M.h. is 94%.

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 dilutionsin 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	MXF	LVX	ERY	TET	DOX	MXF	LVX	CLI
Agree-ment	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); 1discrepancy 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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