MYCOFAST® *RevolutioN* ATB+

Urogenital Mycoplasma Diagnosis
Detection
Enumeration
Identification
Susceptibility testing
25 tests (REF 00070)

CPB 0409_EN-2023-08
For *in vitro* diagnostic use only, for professional use only

I - INTENDED USE

MYCOFAST *RevolutioN* ATB+ 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* ATB+ can be used to determine the susceptibility of U.u. and M.h. to 11 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-gonococcal 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* ATB+ 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 liberation 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 – REACTIFS

Description	Amount
UMMt: Vial of 3 mL mycoplasma broth with antimicro bial agents and preservative solution. pH: 6.0 ± 0.1	25
MYCOFAST® *RevolutioN* ATB+: 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 ATB+ tray, in each of the 24 wells, contains the dehydrated mycoplasma culture medium (foal serum, yeast extract, cysteine, arginine, urea, phenol red, antibiotics, pH: 6.1 ± 0.1) and 1 to 4 concentrations of 11 antibiotics:

Well	20	19	18	17	16	15	14	13	12	11
Antibiotic	ROX	TEL	CLI		ERY		TET			
Concentration	2	4	0.5	0.25	16	8	8	4	2	1

Well	Antibiotic	Concentration		Concentration	Antibiotic	Well
24	PRI	2	MYCOFAST® *RevolutioN* ATB+	1	OFX	22
23	JOS	2		2	MIN	21

Well	1	2	3	4	5	6	7	8	9	10
Content	Uu 10^3	Uu $\geq 10^4$	Mh $\geq 10^4$	1	2	4	0.25	0.5	2	4
Antibiotic				LVX			MXF			

Wells 1/2: Enumeration for U.u. between 10^3 and $\geq 10^4$ CCU/mL (buffered solution and lincomycin included to inhibit the growth of M.h.) (in blue)
Well 3: Enumeration of M.h. at $\geq 10^4$ CCU/mL (in red)
Wells 4/5/6: Evaluation of mycoplasma susceptibility to Levofloxacin (LVX) at 1/2/4 µg/mL
Wells 7/8/9/10: Evaluation of mycoplasma susceptibility to Moxifloxacin (MXF) at 0,25/0,5/2/4 µg/mL
Wells 11/12/13/14: Evaluation of mycoplasma susceptibility to Tetracycline (TET) at 1/2/4/8 µg/mL
Wells 15/16: Evaluation of mycoplasma susceptibility to Erythromycin (ERY) at 8/16 µg/mL (in red)
Wells 17/18: Evaluation of mycoplasma susceptibility to Clindamycin (CLI) at 0,25/0,5 µg/mL (in blue)
Wells 19: Evaluation of mycoplasma susceptibility to Telithromycin (TEL) at 4 µg/mL
Wells 20: Evaluation of mycoplasma susceptibility to Roxithromycin (ROX) at 2 µg/mL
Wells 21: Evaluation of mycoplasma susceptibility to Minocycline (MIN) at 2 µg/mL
Wells 22: Evaluation of mycoplasma susceptibility to Ofloxacin (OFX) at 1 µg/mL
Wells 23: Evaluation of mycoplasma susceptibility to Josamycin (JOS) at 2 µg/mL
Wells 24: Evaluation of mycoplasma susceptibility to Pristinamycin (PRI) at 2 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 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 collecting 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 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.
The UMMt medium may be stored temporarily (3 months) at room temperature but is more stable at 2-8°C.
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) 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:

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 reading in 48 hours of liquid sampling having a negative test in 24 hours, only make the presence of the detected mycoplasma without counting.
For the interpretation of the results refer to the results sheet.

10.2.1 Enumeration (wells 1, 2 and 3)

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

1	U.u. 10^3 CCU/mL
1 and 2	U.u. ≥ 10^4 CCU/mL
3	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 ≥10^4 CCU/mL in an cervicovaginal specimen is abnormal (1, 3).

10.2.2 Susceptibility testing (wells 4 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) for levofloxacin, moxifloxacin, clindamycin, tetracycline, erythromycin and telithromycin.
For the other antibiotics, there are no critical concentrations defined by the CLSI.

Interpretation criteria of MIC in µg/mL (interpretation criteria defined by the CLSI):
The strain is said to be **susceptible** when its growth is inhibited by the critical concentration or two critical concentrations of the antibiotic.
The strain is said to be **resistant** if there is:
1/ growth of the strain with the antibiotic tested at a single concentration.
2/ growth at low concentration or at both concentrations with two antibiotic concentrations.

Class	Antibiotic	U.u..		M.h.		Comments
		S	R	S	R	
Quinolones	Levofloxacin*	≤2	≥4	≤1	≥2	
	Moxifloxacin*	≤2	≥4	≤0.25	≥0.5	
	Ofloxacin	≤1	>1	≤1	>1	
Lincosamides	Clindamycin*	/	/	≤0.25	≥0.5	U.u. is naturally resistant to Clindamycin
Tetracydines	Tetracydine*	≤1	≥2	≤4	≥8	Strains susceptible to tetracycline are also susceptible to doxycycline
	Minocycline	≤2	>2	≤2	>2	
Macrolides	Erythromycin*	≤8	≥16	/	/	Strains susceptible to erythromycin are also susceptible to azithromycin M.h. is naturally resistant to Ery-thromycine
	Roxithromycin	≤2	>2	/	/	M.h. is naturally resistant to Roxy-thromycin
	Josamycine	≤2	>2	≤2	>2	
Ketolides	Telithromycin*	≤4	/	≤4	/	
Streptogramines	Pristinamycin	≤2	>2	≤2	>2	

(*interpretation criteria defined by the CLSI)

M. hominis is naturally resistant to 14 and 15 carbon macrolides, including erythromycin and roxythromycin, but is susceptible to 16 carbon macrolides such as josamycin.
U. urealyticum is naturally resistant to lincosamides (clindamycin).
In some patient populations, tetracycline resistance is as high as 45% for U.u. and 39.6% for M.h. (2).
Resistance to quinolones (U.u. and M.h.) (5, 6) and clindamycin (M.h.) has been described but the prevalence is not known.

Help with interpretation:

Susceptibility testing for U.u.

ATB*	LVX				MXF					TET					ERY		
CONC* (µg/mL)	1	2	4	int*	0.25	0.5	2	4	int*	1	2	4	8	int*	8	16	int*
Profile	-	-	-	S	-	-	-	-	S	-	-	-	-	S	-	-	S
	+	-	-	S	+	-	-	-	S	+	-	-	-	R	+	-	R
	+	+	-	R	+	+	-	-	S	+	+	-	-	R	+	+	R
	+	+	+	R	+	+	+	-	R	+	+	+	-	R	/	/	/
	/	/	/	/	+	+	+	+	R	+	+	+	+	R	/	/	/

*ATB= Antibiotics, *CONC= Concentrations, *INT= Interpretation

Susceptibility testing for U.u.

ATB*	TEL		ROX		MIN		OFX		JOS		PRI	
CONC* (µg/mL)	4	int*	2	int*	2	int*	1	int*	2	int*	2	int*
Profile	-	S	-	S	-	S	-	S	-	S	-	S
	+	/	+	R	+	R	+	R	+	R	+	R

Susceptibility testing for M.h.

ATB*	LVX				MFX					TET					CLI		
CONC* (µg/mL)	1	2	4	int*	0.25	0.5	2	4	int*	1	2	4	8	int*	0.25	0.5	int*
Profile	-	-	-	S	-	-	-	-	S	-	-	-	-	S	-	-	S
	+	-	-	R	+	-	-	-	R	+	-	-	-	S	+	-	R
	+	+	-	R	+	+	-	-	R	+	+	-	-	S	+	+	R
	+	+	+	R	+	+	+	-	R	+	+	+	-	R	/	/	/
	/	/	/	/	+	+	+	+	R	+	+	+	+	R	/	/	/

Susceptibility testing for M.h.

ATB*	TEL		ROX		MIN		OFX		JOS		PRI	
CONC* (µg/mL)	4	int*	2	int*	2	int*	1	int*	2	int*	2	int*
Profile	-	S	naturally resistant		-	S	-	S	-	S	-	S
	+	/			+	R	+	R	+	R	+	R

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 vial (3 mL) 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 plate by re-isolating from the original UMMt medium stored at 2-8°C (§ 9.1). 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 from 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 ou *M. hominis* ATCC 23114) previously calibrated at 10^{4-5} CCU/mL.
Inoculate the MYCOFAST *RevolutioN* ATB+ tray and perform the test as indicated in these instructions (§9 and 10).

Expected results (ATCC):

MYCOFAST *RevolutioN* ATB+

	U.u. 10^3	U.u.≥ 10^4	M.h. ≥10^4	LVX	MXF	TET	ERY
Strain U.u. ATCC 27815	+	+	-	S	S	S/R	S
Strain M.h. ATCC 23114	-	-	+	S/R	S	S	R

	CLI	TEL	ROX	MIN	OFX	JOS	PRI
Strain U.u. ATCC 27815	R	S	S/R	S	S/R	S	S
Strain M.h. ATCC 23114	S	S/R	R	S	S	S	S

13 - LIMITATION OF THE METHOD

Some bacteria that are present in quantities of >10^{6-7}CFU/mL and containing 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 ≥7) may cause the UMMt 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 ≤5.5) 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* ATB + 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 – Enumeration

% of overall agreement	U.u.	M.h.	U.u./M.h.
Isolated strains (threshold ≤10^3 UCC/mL) (see § 14.1.1)	97,7	NA	NA
Isolated strains (threshold ≥10^4 UCC/mL) (see § 14.1.1)	96,5	98,9	97,8
Vaginal samples	100	95,7	97,8
Urinary clinical specimens	96,6	97,7	97,1

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 several dilutions (85 tests in total).
The results obtained were compared with those obtained with a micro dilution enumeration method.
For interpretation with a pathological threshold set at 10^3 CCU/mL; the overall agreement for U.u. is 97.7% (we recorded 2 false positives at 10^3 CCU/mL with a micro dilution enumeration method).
For interpretation with a pathological threshold of 10^4 CCU/mL; the overall agreement for U.u. is 96.5% (we recorded 3 false positives at 10^3 CCU/mL with a micro dilution enumeration method). The overall agreement for M.h. is 98.9% (we have identified 1 false positive at 10^3 CCU/mL with a micro dilution enumeration method).
The overall U.u. and M.h. agreement is 97.8%.

14.1.2 Clinical samples/Clinical strains

An initial comparative study was performed using vaginal clinical specimens (n =23) on dry swabs. The results obtained with MYCOFAST RevolutioN ATB + were compared with the micro dilution enumeration method.
The overall agreement for U.u. is 100%; for the M.h. the overall agreement/concordance is 95.7% (we identified 1 false positive at 10^2 CCU/mL with liquid micro dilution enumeration method).

A second comparative study was performed on urinary clinical specimens (n=88).
Results were read and interpreted after 48 hours incubation if the test was negative within 24 hours. The sole presence of mycoplasma with no enumeration was reported, as recommended for liquid samples.
The results obtained with MYCOFAST *RevolutioN* ATB+ were compared with those obtained with liquid micro dilution enumeration method.
The overall agreement/concordance for U.u. is 96.6% (we identified 1 false negative at 10^4 CCU/mL with liquid micro dilution enumeration method), and 2 false positives at 10^2 CCU/mL with liquid micro dilution enumeration method).)
The overall agreement for M.h. is 97.7% (we identified 2 false positives at 10^2 CCU/mL with the routine laboratory method).
The overall agreement for U.u. and M.h. is 97.1%.

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 ATB + method.
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.
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 sensitive (S) or resistant (R) according to CLSI recommendations.
The overall agreement for *Ureaplasma urealyticum* / *Ureaplasma parvum* is: 93.8% (394/420).
The overall agreement for *Mycoplasma hominis* for rates at 10^4-10^5 CCU/mL is: 93.4% (227243).

	***Ureaplasma urealyticum / parvum* (n=42)**									
agreement	**TET**	**MIN**	**MXF**	**LVX**	**OFX**	**ERY**	**JOS**	**PRI**	**TEL**	**ROX**
	39	**38**	**37**	**40**	**34**	**41**	**42**	**42**	**42**	**39**
ME	**3**	**4**	**4**	**2**	**4**	**1**	**0**	**0**	**0**	**0**
VME	**0**	**0**	**1[a]**	**0**	**4[b]**	**0**	**0**	**0**	**0**	**3[c]**

ME: Major Error, VME : Very Major Error

[a] : 1 discrepancy at 10^4 CCU/mL (reference MIC at 4 µg/mL)
[b] : 1 discrepancy at 10^3 CCU/mL (reference MIC at 2 µg/mL), 1 discepancy at 10^4 CCU/mL (reference MIC at 1 µg/mL), 1 discrepancy at 10^5 CCU/mL (reference MIC at 1 µg/mL), 1 discrepancy at 10^5 CCU/mL (reference MIC at 2 µg/mL)
[c] : 1 discrepancy at 10^3 CCU/mL (reference MIC at 2 µg/mL), 1 discrepancy at 10^4 CCU/mL (reference MIC at 2 µg/mL), 1 discrepancy at 10^5 CCU/mL (reference MIC at 4 µg/mL).

	***Mycoplasma hominis* (n=27)**								
agreement	**TET**	**MIN**	**MXF**	**LVX**	**OFX**	**JOS**	**PRI**	**TEL**	**CLI**
	26	**26**	**27**	**27**	**26**	**27**	**27**	**14**	**27**
ME	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**13**	**0**
VME	**1[a]**	**1[b]**	**0**	**0**	**1[c]**	**0**	**0**	**0**	**0**

[a] : discrepancy at 10^4 CCU/mL (reference MIC at >32 µg/mL)
[b] : discrepancy at 10^5 CCU/mL (reference MIC at 4 µg/mL)
[c] : discrepancy at 10^5 CCU/mL (reference MIC at 2 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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