Mycoplasma IES

IVD For *in vitro* diagnostic and professional use only

2℃ 8℃ Store at 2-8 ℃.

CE

Introduction

Mycoplasma is one of the main pathogens which lead to NGU (nongonoccal urethritis), cervical, pelvic inflammatory disease, orchitis, epididymitis etc., and can cause infertility to men and women. These pathogens can attack and destroy genitourinary epithelial cells, cause infection of the AIDS and other sexually transmitted diseases. Clinical sexually transmitted diseases can be caused by Mycoplasma (mainly by UU and MH). Its occurrence has been presenting an up-trend. Antibiotic resistance is becoming more and more severe due to the misuse of antibiotics, which seriously endanger mankind's health. The key to the treatment and prevention of the spread of Mycoplasma is timely and accurate diagnosis. Mycoplasma cultivation is currently still being recognized as a reliable method to diagnose the Mycoplasma infection.

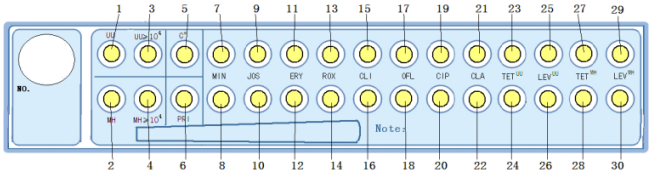
Measurement Principle

Mycoplasma kit is based on cultivation and biochemical reactions. The mixed medium is prepared by mixing the freeze-dried powder and the diluent. After Mycoplasma has been cultivated, urea can be decomposed by urease in UU and release NH_3[3]; and arginine can be decomposed by arginase in MH and release NH_3. NH_3 increases the pH of the liquid medium, the result is judged according to the color change of the indicator. The strip contains 11 antibiotics. If Mycoplasma is sensitive to antibiotic, the activity of enzyme is inhibited, so there is no change in color.

Components

Strip	20
Freeze-dried Powder	20 vials × 1.3 ml
Diluent	20 vials × 4 ml
Mineral Oil	1 vial × 28 ml

1. Strip



The strip contains 30 wells and is divided to 3 sections.

1.1 Culture and identification (wells no. 1, 2, 5)

Well no. 5 (C+): positive control.
Well no. 1 (UU): identification of *U. urealyticum.*
Well no. 2 (MH): identification of *M. hominis*

Wells	Tests	Principal Substrate
No.5	C+	N/A
No.1	UU	Lincomycin
No.2	MH	Erythromycin

1.2 Enumeration (wells no. 3 and 4)

Well no. 3 (UU ≥ 10^4): enumeration of *U. urealyticum.*
Well no. 4 (MH≥10^4): enumeration of *M. hominis*

Wells	Tests	Principal substrate
No.3	UU≥10^4	Lincomycin and inhibition agent
No.4	MH≥10^4	Erythromycin and inhibition agent

1.3 Susceptibility tests (wells no. 6 to 30)

These wells are used to test the susceptibility of the strain with 11 antibiotics.

Wells	Antibiotics and Abbreviations		Concentrations mg/l	
No.6	Pristinamycin	PRI	2	
No.7 and 8	Minocycline	MIN	2	8
No.9 and 10	Josamycin	JOS	2	8
No.11 and 12	Erythromycin	ERY	8	16
No.13 and 14	Roxithromycin	ROX	1	4
No.15 and 16	Clindamycin	CLI	0.25	0.5
No.17 and 18	Ofloxacin	OFL	1	4
No.19 and 20	Ciprofloxacin	CIP	1	2
No.21 and 22	Clarithromycin	CLA	1	4
No.23 and 24	Tetracycline	TET^{UU}	1	2
No.25 and 26	Levofloxacin	LEV^{UU}	2	4
No.27 and 28	Tetracycline	TET^{MH}	4	8
No.29 and 30	Levofloxacin	LEV^{MH}	1	2

Some of the antibiotics are coated according the table below.

Antibiotics		UU		MH		Comments
Class	Drug	S	R	S	R	
Quinolones	Levofloxacin	2	4	1	2	
Macrolides	Erythromycin	8	16	-	-	Organisms susceptibility to erythromycin will also be susceptibility to azithromycin
Lincosamides	Clindamycin	-	-	0.25	0.5	
Tetracycline	Tetracycline	1	2	4	8	Organisms susceptibility to tetracycline will also be susceptibility to doxycycline

Note:

S denotes susceptible.
R denotes resistant.
This table is based upon CLSI Document M43-A, Methods For Antimicrobial Susceptibility Testing For Human Mycoplasmas.

2. Freeze-dried Powder

20 vials each containing 1.3 ml of peptone of bovine origin and beef heart infusion. Contains inhibition agent. The growth of interfering organisms could be inhibited while the growth of Mycoplasma could be promoted.

3. Mineral Oil

1 vial containing 28 ml of liquid paraffin.

4. Diluent

20 vials each containing 4 ml of solution which is used to dissolve the freeze-dried powder.

After reconstitution of the freeze-dried powder with the diluent, the composition is the following:

Formula in g/l purified water

Peptone of bovine origin.....	7.3 g
Yeast extract.....	2.5 g
Beef heart infusion.....	6.6 g
Urea.....	3.6 g
Arginine hydrochloride.....	3.6 g
Salt-mixture.....	797ml
Horse serum.....	181 ml
Phenol red.....	6 ml
Growth factors mixture.....	7 ml
Antibiotic mixture.....	9 ml

the pH is 6.3 ± 0.3

5. 1 copy of instruction for use
6. 20 sheets of result paper

Materials required but not provided:

1. Sample collection swabs.
2. 20 pipette tips.
3. Bacteriology incubator (36 ℃, 37 ℃, 38 ℃).

Warnings and Precautions

1. For *in vitro* professional use only. Can not be reused.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Wear disposable gloves when dealing with samples and reagents. Wash hands after operations.
5. Conduct the assay away from bad ambient conditions. e.g. ambient air containing strong acid, strong alkali or volatile gas and so on.
6. The growth of Mycoplasma in the culture broth would not generate turbidity. This assay has adopted a unique method to effectively inhibit the growth of irrelevant bacteria (including the inhibition of Streptococcus pneumoniae, Staphylococcus epidermidis, Salmonella enteritidis, Micrococcus luteus, Staphylococcus saprophyticus, Pseudomonas aeruginosa, Clostridium sporogenes, Candida guilliermondii, Candida glabrata, Candida tropicalis, Candida krusei, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Enterococcus faecium, Neisseria gonorrhoeae, Streptococcus pyogenes and Pneumonia Kleber, etc). If the mixed medium occasionally displays turbidity and turns red, this does not indicate a positive result.
7. After adding the mixed medium with added samples to each susceptibility well, if it is observed that the color of the mixed medium in all the wells becomes darker or turns to light red, this may be due to the biased alkalinity of samples from patients under pathological conditions. In this case, it is recommended to retest secretion samples from the patients.
8. When testing the antibiotic susceptibility of positive samples validated by normal growth medium, add 50 µl of the cultured positive sample to the mixed medium from this kit and follow the assay procedures mentioned below except that the Mycoplasma must be reinoculated when the bottom of the vial turns red. Otherwise the pH increases, the Mycoplasma die very rapidly and the successful reinoculation rate would be low.
9. Consider the samples, reagent vials and strips for testing as potentially infectious material and deal them in accordance with biosafety laboratory practices.
10. Do not use reagents after expiry date.
11. Do not mix or use components from kits with different batch codes.
12. Do not use vials with turbid appearance.
13. Do not use strips which have been damaged: cupules deformed, desiccant sachet open，and aluminum foiled pouch broken.
14. The performance data presented were obtained using the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.
15. Since Mycoplasma have a high affinity for mucus cell membranes, it is important to thoroughly scrape the mucosa so as to collect as many cells as possible.
16. Collect the sample before administering any antibiotic treatment.
17. A standardized technique must be used to prevent contamination by other microorganisms.
18. A sample cannot be considered as negative before 24 hours of incubation.
19. If the sample titer is low, the strip cupules may not change color or the color change may be inconsistent.
20. Enumeration in the tests carried out on the strip can only give an indication of the titer. The exact titer can be determined on agar.
21. The antibiotics are tested on the sample, without taking into account the Mycoplasma titer of the sample. In the case of low titers, the real susceptibility of the strain may be different from the result obtained with the strip.
22. A result which is negative at the lowest concentration of an antibiotic, and positive at the highest concentration is meaningless. In this case, perform the test again.
23. If the outer packaging is damaged, it is still appropriate to use the kit. However, if the immediate packaging is damaged or the analytical performance is changed, do not use the kit.

Storage

1. Store all components at 2-8 ℃.
2. Use the strip within 8 hours once unwrapped.
3. Use the culture medium, after the diluent and freeze-dried powder are mixed together within 72 hours.
4. Use the inoculated medium within 8 hours at 18-28 ℃ or within 48 hours at 2-8 ℃.
5. The mineral oil may be used until the labeled expiry date once opened.
6. During transportation, store the kit at a low temperature and away from sunlight. Ship the kit in cold chain.

Sample

1. If the sample is to be inoculated to the mixed medium, inoculate within 4 hours. For a special case, store the sample at 2-8 ℃ and inoculate within 24 hours.
2. If the sample is to be collected and transported with a UTM swab, store the UTM swab sample at room temperature (18-25 ℃) within 24 hours, for longer storage, the UTM sample should be stored at 2-8 ℃ up to 48 hours.
3. If the sample is to be inoculated to the diluent (the inoculated diluent can be used as transport medium), store the inoculated diluent at room temperature (18-25 ℃) within 24 hours, for longer storage, the inoculated diluent should be stored at 2-8 ℃ up to 48 hours.
4. For Endocervical and urethral samples, Use only a Dacron or rayon or cotton swab, or a cytobrush to collect samples; collect after the exocervix, or the meatus have been carefully cleansed with a first swab.
   Note: Mycoplasmas adhere strongly to mucous cells. The mucous lining should be well scraped to obtain a rich sample. Inoculate to the diluent or mixed culture medium and dispose of the swab.
5. For urine samples, collect midstream of urine in a sterile bottle. Inoculate 500 µl of the homogenized urine to the diluent or mixed culture medium with a pipette.
6. For other types of samples, e.g. semen or other less frequent liquid samples are collected in a sterile bottle. Inoculate 25 µl of the semen to the diluents or mixed culture medium.

Reagent Preparation

1. Bring all reagents to room temperature (18-25 ℃) prior to use.
2. Adjust the incubator at 37 ℃.

Measurement Procedure

1. **<u>If the Sample is Collected and Inoculated at the Same Place</u>**
   a. Add the diluent completely to the freeze-dried powder, and shake to mix completely.
   b. Inoculate the swab sample or **500 µl of the midstream urine sample or 25 µl of the semen sample** to the mixed medium. Place the lid on the vial, and shake to mix completely.
   c. Add **100 µl of the inoculated medium** to all the wells on the strip. Shake gently to dissolve the coated materials.
   d. Add **1 drop of mineral oil** to each well.
   e. Cover the strip. Incubate at 36-38 ℃ for 24 hours.

2. **<u>If the Sample is Collected and Inoculated at Different Places and Transported in the Diluent</u>**
   a. At the place of sample collection, add the swab sample or **500 µl of the midstream urine sample or 25 µl of the semen sample** to the diluent. Then send the inoculated diluent to the place where the test is to be conducted.

b. Add the inoculated diluent to the freeze-dried powder. Place the lid on the vial, and shake to mix completely.
c. **Add 100 µl of the inoculated medium** to all the wells on the strip. Shake gently to dissolve the coated materials.
d. **Add 1 drop of mineral oil** to each well.
e. Cover the strip. Incubate at 36-38 ℃ for 24 hours.

3. <u>If the Sample is Collected and Inoculated at Different Places and Transported in the UTM</u>

a. Add the diluent completely to the freeze-dried powder.
b. Inoculate **400 µl of the UTM sample** to the mixed medium. Place the lid on the vial, and shake to mix completely.
c. Add **100 µl of the inoculated medium** to all the wells on the strip. Shake gently to dissolve the coated materials.
d. Add **1 drop of mineral oil** to each well.
e. Cover the strip. Incubate at 36-38° C for 24 hours.

Measurement Results

Read the color change on the strip. If the color turns from yellow to red or peach blow, it implicates the growth of Mycoplasma; if the color doesn't change, it could be deemed to be negative or sensitive to antibiotics; Seldom, the culturing medium turns light red (i.e. the color does not change evidently) after being cultivated for 24 hours. In this case, it is recommended to extend the culture time by another 12-24 hours. (Because the patient may be infected by Mycoplasma recently, in the recovery period or under antibiotic treatment such that there is only very little amount of Mycoplasma in the sample or the Mycoplasma is inhibited by antibiotics. Consequently, the color change is not evident.). The strain is susceptible when it is inhibited by both the two concentrations of the antibiotics, is intermediate when it is inhibited by the higher concentration while not inhibited by the lower concentration, is resistant when it is neither inhibited by the lower concentration nor the higher concentration.

The table above is an illustration of how to read the results according to the color of each well on the strip.

Note: the pathological thresholds usually quoted for U. urealyticum are: ≥10^4 CCU/ml for an urethral sample, and UU positive in a urine stream or sperm sample, no matter the quantity is ≥10^4 CCU/ml or not. The threshold for M. hominis is ≥10^4 CCU/ml in an endocervical sample. Because pristinamycin is coated at only one concentration, the strain is resistant when the well turns red while it is susceptible when the well stays at yellow. According to CLSI guideline, the susceptibility to erythromycin is also applicable to azithromycin while the susceptibility to tetracycline is also applicable to doxycycline.

	Culture and Identification			Enumeration		Susceptibility tests (mg/l)											
Wells	C+	UU	MH	UU≥10^4	MH≥10^4	PRI	MIN	JOS	ERY	ROX	CLI	OFL	CIP	CLA	TET^{UU}	LEV^{UU}	TET^{MH}	LEV^{MH}
						2	2/8	2/8	8/16	1/4	0.25/0.5	1/4	1/2	1/4	1/2	2/4	4/8	1/2
Negative	Yellow			Yellow		Yellow	Yellow											
Positive	Yellow or Turns to red			Yellow or Turns to red		Yellow or Turns to red	Yellow or Turns to red											
Notes	UU and/or MH positive	UU	MH	UU≥104	MH≥10^4	A change of color indicates resistant to antibiotic; no color change indicates susceptible to antibiotic	No color change in both of the wells indicates susceptible to antibiotic; a color change in the upper well and no color change in the lower well indicates intermediate to antibiotic; a color change in both of the wells indicates resistant to antibiotic.											

Control Procedure

The recommended control requirement for this assay is to purchase reference strains (UU (ATCC® 27813) and MH (ATCC® 15488)) separately. Culture ATCC® 27813 in the mixed medium. Incubate until the culture medium turns to light red then perform a subculture to another vial of mixed medium and incubate until culture medium turns to light red. Carry out a 1000 folds dilution of this culture medium with sterile saline solution and add 100 µl to a new vial of mixed medium. Inoculate the strip with this final culture. The result is valid if the color of C+, UU, UU ≥ 10^4, CLI (both low and high concentrations), OFL (low concentration) and CIP (both low and high concentrations) wells turns from yellow to red or peach blow. Test ATCC® 15488 in the same operation as above. The result is valid if the color of C+, MH, MH ≥ 10^4, ERY (both low and high concentrations), CLA (both low and high concentrations) and ROX (both low and high concentrations) wells turns from yellow to red.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. A very small number of alkaline samples may cause the culture medium turn red directly because this test is based on culture and biochemical reactions and the resulting increase in pH leads to the change in the color of the mixed medium.
3. Since the clinical abuse of antibiotics leads to the emergence of a small number of drug-resistant strains, a very small number of false positive results might be obtained despite the adoption of various antibiotics in the culture broth to inhibit irrelevant bacteria. Hence we recommend confirming the positive samples with a Mycoplasma agar plate whenever practicable.

Performance Characteristics

1. <u>Performance with strains</u>

For the mixed medium, 12 pure mycoplasma strains inoculated at 2 dilutions as well as 3 mixtures of UU and MH at 2 dilutions were detected positive, whatever the dilution. In addition, of the 19 interferent strains in urogenital samples at 0.5 McFarland, 100 µl from each were taken and inoculated. The results were all negative.

For the strip, 3 pure mycoplasma strains inoculated at 2 dilutions as well as 6 mixtures of UU and MH at 2 dilutions were correctly identified by the strip. 12 pure mycoplasma strains were cultured in the mixed medium until the color turns to light red and then were diluted at the concentration of 10^4 CCU/ml and were tested. The corresponding UU≥10^4 or MH≥10^4 wells turned to red. 3 pure UU strains at 2 dilutions were tested for a total of 6 times. The color of CLI well (both low and high concentrations) turned to red for 6 tests, the color of CIP well (low concentration) turned to red for 3 tests, the color of CIP well (both low and high concentrations) turned to red for 3 tests, the color of TET^{UU} wells (both low and high concentrations) turned to red for 4 tests, the color of OFL well (low concentration) turned to red for 3 tests, the color of OFL well (both low and high concentrations) turned to red for 1 test, the color of MIN well (low concentration) turned to red for 1 test, the color of MIN well (both low and high concentration) turned to red for 3 tests, the color of PRI, ERY, ROX, JOS, CLA, LEVUU wells (both low and high concentrations) remained yellow for 6 tests. 3 pure MH strains at 2 dilutions were tested for a total of 6 times. The color of ERY, CLA and ROX wells (both low and high concentrations) turned to red, the color of OFL wells (both low and high concentrations) turned to red for 4 tests, the color of LEV^{MH} wells (both low and high concentrations) turned to red for 4 tests, the color of CIP well (low concentration) turned to red for 1 test, the color of CIP well (both low and high concentrations) turned to red for 3 tests, the color of MIN, PRI, JOS, CLI, TET^{MH} wells (both low and high concentrations) remained yellow for 6 tests.

2. Measurement Accuracy by Correlation

A study was performed where samples were tested using this assay and two other CE marked assay. When two of the three assays generated a positive result, the sample is true positive. Otherwise, the sample is negative. This is called amplified gold standard. The comparison between this assay and the amplified gold standard is presented below.

The comparison between this assay and the amplified gold standard

		Amplified gold standard		total
		positive	negative	
this assay	positive	48	0	48
	negative	0	91	91
	total	48	91	139

In x^2 method, P>0.05, there is no obvious difference between the two methods.

Literature References

1. Núñez-Calonge R, Caballero P, Redondo C, et al. Ureaplasma urealyticum reduces motility and induces membrane alterations in human spermatozoa. Hum. Reprod. 1998;13(1O):2756-2761.
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3. Murray P. Manual of clinical microbiology. 9th ed. Washington D.C.: ASM Press; 2007.
4. Clinical and Laboratory Standards Institude（CLSI）. 2011 Methods For Antimicrobial Susceptibility Testing For Human Mycoplasmas; Approved Guideline.CLSI Docunment M43-A.Vol. 31-№19.

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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry