1. INTRODUCTION

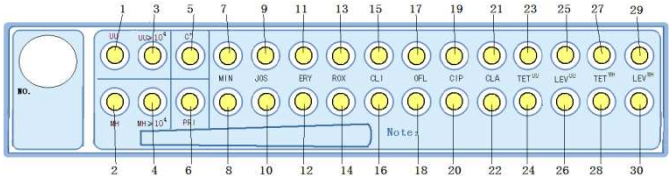
Mycoplasma is one of the main pathogens which lead to NGU (nongonococcal urethritis), cervical, pelvic inflammatory disease, orchitis, epididymitis *etc.*, and can cause infertility to men and women[1]. These pathogens can attack and destroy genitourinary epithelial cells, cause infection of the AIDS and other sexually transmitted diseases. Clinical sexually transmitted diseases can be caused by *Mycoplasma* (mainly by *Urea urealyticum* UU and *Mycoplasma hominis*). 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[2]. 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.

2. PRINCIPLE

MYCOPLASMATEST 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. Strip contains 11 antibiotics and each one has two concentrations, If *Mycoplasma* is sensitive to antibiotic, the activity of enzyme is inhibited, so there is no change in color.

3. MATERIAL REQUIRED

1. 20 strips each containing:



► 1 well C+: Positive Control (n°5) coated with arginin, urea and some Mycoplasma growth activator.

► 29 wells, among which the UU well (n°1) is coated with lincomycin, MH well (n°2) is coated with erythromycin, UU≥10^4 well (n°3) is coated with lincomycin and inhibition agent, MH≥10^4 well (n°4) is coated with erythrocin and inhibition agent, then 25 wells are coated with 11 antibiotics at 2 concentrations, except PRI (see table below) :

Well number	Abreviation	Signification	Low concentration (up well) (mg/L)	High concentration (down well) (mg/L)
6	PRI	pristinamycin	2	/
7, 8	MIN	mincycline	2	8
9, 10	JOS	josamycin	2	8
11, 12	ERY (AZI)	erythromycin (azithromycin)*	8	16
13, 14	ROX	roxithromycin	1	4
15, 16	CLI	clindamycin	0,25	0,5
17, 18	OFL	ofloxacin	1	4
19, 20	CIP	ciprofloxacin	1	2
21, 22	CLA	clarithromycin	1	4
23, 24	TET^{UU} (DOX)	tetracycline (doxycycline)*	1	2
25, 26	LEV^{UU}	levofloxacin	2	4
27, 28	TET^{MH} (DOX)	tetracycline (doxycycline)*	4	8
29, 30	LEV^{MH}	levofloxacin	1	2

Note*: Organisms sensitive to tetracycline are sensitive to doxycycline (DOX) too. Organisms sensitive to erythromycin are sensitive to azithromycin (AZI) too. This information are based upon CLSI Document M43-A, Methods For Antimicrobial Susceptibility Testing for Human Mycoplasmas.

► 1 pipettes tips

2. Freeze-dried Powder

20 vials each containing 1.2 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. Diluent

20 vials each containing 4 ml of solution which is used to dissolve the freeze-dried powder. The culture medium, after the diluent and freeze-dried powder are mixed together, conforms to the formula which is, of each 1000 ml of purified water, there are 7.3 g of peptone of bovine origin, 2.5 g of yeast extract, 6.6 g of beef heart infusion, 3.6 g of urea, 3.6 g of arginine hydrochloride, 797 ml of salt-mixture, 181 ml of horse serum, 6 ml of phenol red, 7 ml of growth factors mixture and 9 ml of antibiotic mixture. The pH is 6.3±0.3.

4. Mineral Oil

1 vial containing 28 ml of liquid paraffin.

5. 1 copy of instruction for use

6. 20 sheets of result paper

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Cotton, polyester sterile, STUART swab, Eswab or UTM swab, for the sample collection.
- Bacteriology incubator (36 °C, 37 °C,38 °C)
- UTM medium (Universal Transport Medium), can be used for transport medium.

5. CONSERVATION AND STABILITY OF THE COMPONENTS

- Store all components at 2-8 °C.
- However the kit can be transported and stored at a temperature between -10 and 37°C during 7 days without the life and qualities of the product are altered. An unopened diluent can be stored at room temperature within one month.
- Use the strip within 8 hours once unwrapped.
- The Mineral Oil may be used until the labelled expiry date once opened.
- Use the culture medium, after the diluent and freeze-dried powder are mixed together within 72 hours.
- Use the inoculated medium within 8 hours at 18-28 °C,or within 48 hours at 2-8 °C.

6. PRECAUTIONS

- For professional use only. Can not be reused.
- 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.
- Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
- Wear disposable gloves when dealing with samples and reagents. Wash hands after operations.
- Conduct the assay away from bad ambient conditions. e.g. ambient air containing strong acid, strong alkali or volatile gas and so on.
- 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.
- 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 other strip wells evidently 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.
- 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. The re-inoculation should be conducted before the bottom of the vial turns red, otherwise the pH will increase, leading to the rapid death of the Mycoplasma and the lowered reinoculation possibility.
- Consider the samples, reagent vials and strips for testing as potentially infectious material and deal them in accordance with biosafety laboratory practices.
- Do not use reagents after expiry date.
- Do not mix or use components from kits with different batch codes.
- Do not use vials with turbid appearance.
- Do not use strips which have been damage: cupules deformed, dessicant sachet open, and aluminium failed pouch broken.
- The performance data presented were obtained using the procedure indicated in this package insert. Any change or modification in the procedure may affect the result.
- 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.
- Collect the sample before administering any antibiotic treatment.

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- A standardized technique must be used to prevent contamination by other microorganisms.
- A sample cannot be considered as negative before 24 hours of incubation.
- If the sample titre is low, the strip cupules may not change color or the color change may be inconsistent.
- Enumeration in the tests carried out on the strip can only give an indication of titer. The exact titre can be determinate on agar.
- The antibiotics susceptibility results of the samples do not take 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.
- A result which is negative at the lowest concentration of an antibiotic, and positive at the highest concentration is meaningless. In this case, perfom the test again.
- If the outer packaging is damaged, it is still appropriate to use the kit. However, if the interior packaging is damaged or the analytical performance is changed, do not use the kit.

7. SAMPLE AND CONSERVATION

7.1 SAMPLE

1. 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 cleaned with a first swab.
   Note: Mycoplasmas adhere strongly to mucous cells. The mucous lining should be well scraped to obtain an abundant amount sample. Inoculate to the diluent or mixed culture medium and dispose the swab.
2. For urine samples, collect midstream of urine (for males) in a sterile bottle. Inoculate 500 µl of the homogenized urine to the diluent or mixed culture medium with a pipette.
3. 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.

7.2. SAMPLE CONSERVATION

1. If the sample is inoculated in the mixed medium, the inoculated medium may be kept for 4 hours at room temperature (18-25°C), or 24 hours at 2-8°C at the most.
2. If the sample is to be inoculate to the diluent (the inoculated diluents can be used as transported medium), store the inoculated diluents at room temperature(18-25 °C) within 24 hours, for longer storage, the inoculated diluent should be stored at 2-8 °C up to 48 hours.
3. If the sample is to be collected and transported with a UTM Swab (Universal Transport Medium), store the UTM swab at room temperature (18-25°C) within 24 hours, for longer storage, the UTM swab sample should be store at 2-8 °C up to 48 hours.

4. PROCEDURE

Bring all reagents to room temperature (18-25°C) prior to use
Adjust the incubator at 37 °C.

<u>If the Sample is Collected and Inoculated at the Same Place</u>

1. Add the diluent completely to the freeze-dried powder, and shake to mix completely.
2. 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.
3. Add 100 µl of the inoculated medium to all the wells on the strip. Shake gently to dissolve the coated materials.
4. Add 1 drop of mineral oil to each well.
5. Cover the strip. Incubate at 36 – 38°C for 24 hours.

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

1. 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.
2. Add the inoculated diluent to the freeze-dried powder. Place the lid on the vial, and shake to mix completely.
3. Add 100 µl of the inoculated medium to all the wells on the strip. Shake gently to dissolve the coated materials.
4. Add 1 drop of mineral oil to each well.
5. Cover the strip. Incubate at 36-38 °C for 24 hours.

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

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

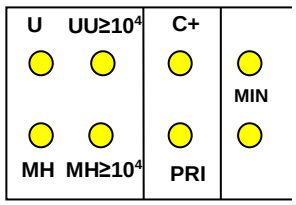
9. RESULTS AND INTERPRETATION

Use one sheet of result paper per test.
Read the color change on the strip.

<u>The well C+ :</u> If the color turns red, it implicates the growth of *Mycoplasma* ; the sample is positive. If the color doesn't change, and stays yellow, it is negative.

<u>The others well :</u> If the color turns from orange to red or peach blow, it implicates the growth of *Mycoplasma*, it is resistant. If the color doesn't change, and stays yellow, 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 below is an illustration of how to read the results according to the color of each well on the strip.
<u>Note:</u> 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.

<u>Example of results:</u>

<table>
<tr>
<td> 
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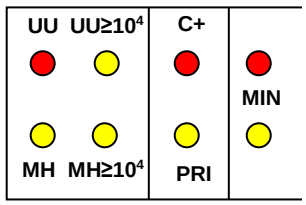
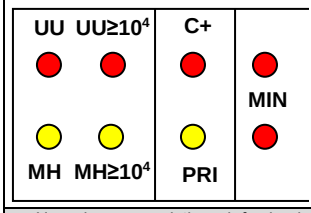
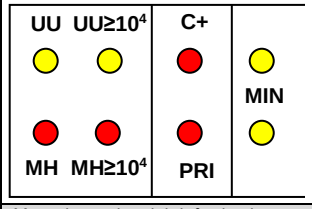
<td> 
</td>
</tr>
<tr>
<td>No Mycoplasma infection</td>
<td>Ureaplasma urealyticum infection
PRI is sensible (S)
MIN is intermediate (I)</td>
</tr>
<tr>
<td> 
</td>
<td> 
</td>
</tr>
<tr>
<td>Ureaplasma urealyticum infection is more than 10^4 CCU/mL
PRI is sensible (S)
MIN is resistant (R)</td>
<td>Mycoplasma hominis infection is more than 10^4 CCU/mL
PRI is resistant (R)
MIN is sensitive (S)</td>
</tr>
</table>

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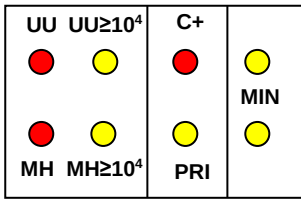
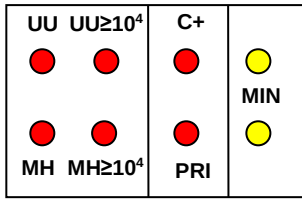
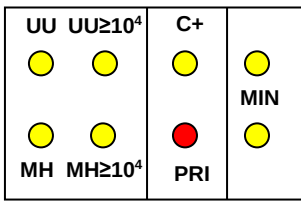
	
Mycoplasma hominis and ureaplasma urealyticum infection PRI is sensible (S) MIN is sensitive (S)	*Mycoplasma hominis* and ureaplasma urealyticum infection are more than 10^4 CCU/mL. PRI is resistant (R) MIN is sensitive (S)
	<u>Sensitive (S):</u> The probability of therapeutic success is acceptable. We have to expect a therapeutic effect in the case of a treatment with usual dose. <u>Intermediate (I):</u> The therapeutic success is unpredictable. <u>Resistant (R):</u> Strong probability of therapeutic failure. We cannot expect a therapeutic effect whatever is the treatment.
Invalid	

10. QUALITY CONTROL

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 orange to red or peachblow. 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 orange to red or peachblow.

Control standards are not supplied with this kit; however, it is recommended that positive and negative controls are tested as a good laboratory practice to confirm the test procedure and to verify proper test performance. Each laboratory has to set up its own planning of controls.

11. LIMITS

- 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.
- 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.
- 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.

12. PERFORMANCES

12.1. Performance with strains

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 interfering 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, LEV^{UU} wells (both low and high concentrations) remained orange 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, TETMH wells (both low and high concentrations) remained orange for 6 tests.

12.2. Measurement Trueness 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.

		Amplified gold standard		total
		positive	negative	
this assay	positive	48	2	50
	negative	0	89	89
	total	48	91	139

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

13. LITERATURE

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.
2. Rylander M, Hallander HO. In vitro comparison of the activity of doxycycline, tetracycline, erythromycin and a new macrolide, CP 62993, against Mycoplasma pneumoniae, Mycoplasma hominis and Ureaplasma urealyticum. *Scand J Infect Dis Suppl*. 1988;53:12-17.
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-N°19.*

SYMBOLES / SYMBOLS

	Attention, Lire la notice d'utilisation. Attention, see instructions for use	LOT	Numéro du lot Lot number
IVD	Pour diagnostic in vitro. For *in vitro* diagnostic use only		Fabriquant Manufacturer
2°C 30°C	A conserver entre 2-8°C Store between 2-8°C		Ne pas réutiliser Do not reuse
Σ	Nombre de test par kit Tests per kit	REF	Référence catalogue Catalog number
	Date de péremption Expiry		

VERSION 01 – 28/12/2015

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