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Microbiological Assay

REF M0206

20 tests

Mycoplasma TIES

Dehydrated culture medium based assay for the screening, indicative enumeration, identification, typing and antimicrobial susceptibility testing of UP (Ureaplasma parvum), UU (Ureaplasma urealyticum) and MH (Mycoplasma hominis) in human genitourinary tract. The selection of antimicrobials is partially based on CLSI (Clinical and Laboratory Standards Institute) recommendations.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		date of manufacture

EC REP
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CE IVD

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Introduction

Ureaplasmas spp. and *M.hominis (MH)* can infect the human genitourinary tract[1], and cause human non-gonococcal urethritis (NGU), cervicitis, pelvic inflammatory disease, infertility, adverse pregnancy, etc. These pathogens can invade and destroy urogenital tract mucosal epithelial cells, more likely to cause secondary infection of other venereal diseases and AIDS[2-4]. However, there are many controversial conclusions about *Ureaplasma urealyticum* as a pathogen of genitourinary tract infection, which is related to the fact that *Ureaplasma urealyticum* contains two biotypes. IUMS classified the two biotypes of *Ureaplasma* into *UP (Ureaplasma parvum)* and *UU (Ureaplasma urealyticum)*.[5] Studies have shown that *UU* and *UP* have different pathogenicity in different populations, and *UP* is considered as a normal flora in NGU, lower genital tract infection, pelvic inflammatory disease and other populations.[6] The proportion of *UU* was significantly lower than that of *UP*.

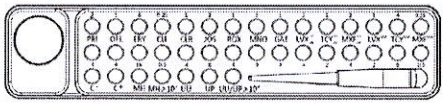
Although there are some differences among different reports, the overall ratio of the former to the latter is about 1:3, which means that for non-pregnant or pregnant people, about 75% of positive *Ureaplasma* tests regardless of type are caused by *UP*, which is considered to be the normal flora. Therefore, the classification of *UU* and *UP* has important clinical significance.

Measurement Principle

Mycoplasma TIES includes a selective medium and 20 test strips. The mixed medium is prepared by mixing the lyophilized powder and the diluent. After the sample with mixed medium has been cultured, urea within the culture broth can be decomposed by urease in *Ureaplasma* spp. and release NH_3; arginine within the culture broth can be decomposed by arginase in MH and release NH_3. NH_3 increases the pH of the culture broth; the result is read according to the color change of the indicator. The species *UP (Ureaplasma parvum)* and *UU (Ureaplasma urealyticum)* are distinguished according to their different tolerance to manganese ions. The strip contains 12 antimicrobial agents. If *Mycoplasma* is susceptible to antimicrobial agents, the activity of enzyme would be inhibited; hence there is no change in color.

Components

1. Strip



Total 20 strips each contains 37 wells.

1.1 Culture and identification

Well (C^+): positive control. Well (*UU*), Well (*UP*): identification of *Ureaplasma* spp. Well (*MH*): identification of *M. hominis*.

Wells	Principal Substrate
C^+	N/A
UU	Lincomycin
UP	Lincomycin, Mn^{2+}
MH	Erythromycin

1.2 Enumeration (wells $MH \geq 10^4$ and $UU/UP \geq 10^4$)

Well ($UU/UP \geq 10^4$): enumeration of *Ureaplasma* spp.
Well ($MH \geq 10^4$): enumeration of *M. hominis*

Wells	Principal substrate
$UU/UP \geq 10^4$	Lincomycin and inhibition agent
$MH \geq 10^4$	Erythromycin and inhibition agent

1.3 Susceptibility tests

These wells are used to test the susceptibility of the strain with 12 antimicrobial agents.

Antimicrobials and Abbreviations		Concentrations (μg/mL)	
Pristinamycin	PRI	2	4
Ofloxacin	OFL	1	4
Erythromycin	ERY	8	16
Clindamycin	CLI	0.25	0.5
Clarithromycin	CLR	1	4
Josamycin	JOS	2	8
Roxithromycin	ROX	1	4
Minocycline	MNO	2	8
Gatifloxacin	GAT	1	4
Levofloxacin	LVX^{UU}_{UP}	2	4
Tetracycline	TCY^{UU}_{UP}	1	2
Moxifloxacin	MXF^{UU}_{UP}	2	4
Levofloxacin	LVX^{MH}	1	2
Tetracycline	TCY^{MH}	4	8
Moxifloxacin	MXF^{MH}	0.25	0.5

Some of the antimicrobial agents are coated according the CLSI Document M43-A, Methods for Antimicrobial Susceptibility Testing for Human Mycoplasmas.

2. Lyophilized Powder

20 vials containing peptone and extract powder of bovine origin. 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 minimum 37 mL of liquid paraffin.

4. Diluent

20 vials each containing minimum 4 mL of solution which is used to dissolve the lyophilized powder.

After reconstitution of the lyophilized powder with the diluent, the composition is the following:

Composition in reconstituted lyophilized powder
Yeast extract
Peptone
NaCl
Animal serum
Inhibitor
Indicator
Water
the pH is 6.2 ± 0.5

5. 1 copy of instruction for use

6. 20 sheets of result paper

7. 20 pipette tips

Materials Required but not Provided

1. Sample collection swabs
2. Bacteriology incubator

Warnings and Precautions

1. For professional use only. Cannot 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 adopts 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 culture broth occasionally displays turbidity and turns to red, this does not indicate a positive result.
7. After adding the mixed medium with sample to each well, it is recommended to retest if color in all the wells become darker or turns to light red due to the biased alkalinity of samples from patients under pathological conditions.
8. When testing the antimicrobials susceptibility of positive samples validated by normal growth medium, add 50 μL of the cultured positive sample to the mixed medium 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 lower re-inoculation possibility.
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: wells deformed, desiccant sachet open or 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 antimicrobials 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 wells 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 antimicrobials 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.
22. A result which is negative at the lower concentration of an antimicrobial 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 interior packaging is damaged or the analytical performance is changed, do not use the kit.
24. There is risk to have cross interference between *UU* and *UP* when the concentration of *Ureaplasma* is too high in the sample.
25. It is possible to have the result that when UU/UP well is positive, but both *UU* well and *UP* well are negative. The reason is that the concentration of *Ureaplasma* in the sample is too low to be typed. It is recommended to report *Ureaplasma* positive after 24 hours or extend the culture time to observe the typing result.

Storage

1. Store all components at 2-8 °C.
2. Use the strip within 8 hours once unwrapped.
3. Dispose the mixed medium after 72 hours since the diluent and lyophilized powder are mixed.
4. Use the inoculated broth within 8 hours at 18-25 °C or within 48 hours at 2-8 °C.
5. The mineral oil may be used until the labeled expiry date once opened.
6. During transportation, store the kit at low temperature and away from sunlight.

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 °C 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 °C) within 24 hours, for longer storage, the UTM sample should be stored at 2-8 °C 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 °C) within 24 hours, for longer storage, the inoculated diluent should be stored at 2-8 °C up to 48 hours.
4. For endocervical and urethral samples, use only a dacron or rayon or cotton swab, or a cytobrush to collect, and should be after the exocervix or the meatus have been carefully cleaned.
   Note: *Mycoplasmas* adhere strongly to mucous cells. The mucous lining should be well scraped to obtain an abundant amount of sample. Inoculate to the diluent or mixed medium and dispose 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 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 100 μL of the semen to the diluents or mixed medium.

Assay Preparation

1. Bring all reagents to room temperature (18-25 °C) prior to use.
2. Adjust the incubator to 36-38 °C.

Measurement Procedure

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

i. Add the diluent completely to the lyophilized powder, and shake to mix completely.
ii. Add 100 μL of the culture broth to Well C⁻.
iii. Inoculate the swab sample or 500 μL of the midstream urine sample or 100 μL of the semen sample to the mixed medium. Cap the vial and shake to mix completely.
iv. Add 100 μL of the culture broth to each well on the strip. Gently shake the strip to dissolve the coated materials.
v. Add one or two drops of the mineral oil. (It must cover the entire surface of the liquid within the well; otherwise, the culture broth may evaporate, leading to inaccurate results.)
vi. Cover the strip with a lid. Incubate at 36-38 °C for 24 hours. Then read the results.

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

i. At the place of sample collection, add the swab sample or 500 μL of the midstream urine sample or 100 μL of the semen sample to the diluent, then send the inoculated diluent to the place where the test is to be conducted.
ii. Add the inoculated diluent to the lyophilized powder. Cap the vial and shake to mix completely.
iii. Add 100 μL of the culture broth to each well of the strip. Gently shake the strip to dissolve the coated materials.
iv. Add one or two drops of the mineral oil. (It must cover the entire surface of the liquid within the well; otherwise, the culture broth may evaporate, leading to inaccurate results.)
v. Cover the strip with a lid. Incubate at 36-38 °C for 24 hours, and then read the results.

Note: When the sample is collected and inoculated at different places and transported in the Diluent, the result of Well C⁻ is the same with the result of Well C⁺.

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

i. Add the diluent completely to the lyophilized powder.
ii. Add 100 μL of the culture broth to Well C⁻.
iii. Inoculate 400 μL of the UTM sample to the mixed medium. Cap the vial and shake to mix completely.
iv. Add 100 μL of the culture broth to each well on the strip. Gently shake the strip to dissolve the coated materials.
v. Add one drop of the mineral oil. (It must cover the entire surface of the liquid within the well; otherwise, the culture broth within the well may evaporate, leading to inaccurate results.)
vi. Cover the strip with a lid. Incubate at 36-38 °C for 24 hours, and then read the results.

Measurement Results

Read the color change on the strip. If the color turns from yellow to red, it implicates the growth of *Mycoplasma*; if the color doesn't change, it could be deemed to be negative or susceptible to antimicrobials; in a rare situation, the culture broth turns light red (*i.e.* the color does not change evidently) after being cultured for 24 hours, it is recommended to extend the culture time by another 12-24 hours. (Because the patient may be infected by *Mycoplasma* shortly and in the recovery period or under antimicrobials treatment such that there is little amount of *Mycoplasma* in the sample or the *Mycoplasma* is inhibited by antimicrobials. Consequently, the color change is not evident.). The strain is susceptible when it is inhibited by both concentrations of the antimicrobials; 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 in the next page is an illustration of how to read the results according to the color of each well on the strip.

	Culture, Typing and Identification				Enumeration		Susceptibility tests (μg/mL)													
Wells	C^+	UU	UP	MH	UU/UP≥10^4	MH≥10^4	PRI	OFL	ERY	CLI	CLR	JOS	ROX	MNO	GAT	LVX^{UU}_{UP}	TCY^{UU}_{UP}	MXF^{UU}_{UP}	LVX^{MH}	TCY^{MH}	MXF^{MH}
							2/4	1/4	8/16	0.25/0.5	1/4	2/8	1/4	2/8	1/4	2/4	1/2	2/4	1/2	4/8	0.25/0.5
Negative	Yellow				Yellow		Yellow														
Positive	Yellow turns to Red				Yellow turns to Red		Yellow turns to Red														
Notes	UU, UP and/or MH positive	UU	UP	MH	UU or UP≥10^4	MH≥10^4	No color change in both of the wells indicates susceptible to antimicrobials; the color change in the upper well and no color change in the lower well indicate intermediate to antimicrobials; the color change in both of the wells indicates resistant to antimicrobials.														

Note: The positive result of some *M. hominis* is orange. The pathological thresholds usually quoted for *Ureaplasma* spp. are: ≥10^4 CCU/mL for an urethral sample, and *Ureaplasma* spp. positive in a urine stream or semen 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. According to CLSI guideline, the susceptibility to erythromycin is also applicable to azithromycin and the susceptibility to tetracycline is also applicable to doxycycline.

Control Procedure

The recommended control method for this assay is to purchase reference strains *UU* (ATCC® 33175), *UP* (ATCC® 27813) and *MH* (ATCC® 23114) separately.

Inoculate the mixed medium containing 10^4-10^5 CCU/mL ATCC® 33175 to the C^+, *UU* and *UU/UP*≥10^4 wells. Incubate at 36-38 °C for 24 hours, the color of the C^+, *UU* and *UU/UP*≥10^4 wells should turn to red.

Conduct the same operation with ATCC® 27813, after incubation, the color of the C⁺, UP and UU/UP≥10^4 wells should turn to red.
Inoculate the mixed medium containing 10^4-10^5 CCU/mL ATCC® 23114 to the C⁺, *MH* and *MH*≥10^4 wells. Incubate at 36-38 ℃ for 24 hours, the color of C⁺, *MH* and *MH*≥10^4 wells should turn to orange or red.
Note: According to CLSI M43-A document, the *Ureaplasma* should be incubated for 1 hour after inoculation, the *Mycoplasma hominis* should be incubated for 2 hours after inoculation.

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 small number of alkaline samples may cause the culture broth turn red directly due to the biochemical reaction that the increasing pH leads to the color change of the culture broth.
3. Since the clinical abuse of antimicrobials causes the emergence of a few numbers of drug-resistant strains, false positive results might be obtained despite the adoption of various antimicrobials in the mixed medium to inhibit irrelevant bacteria. Hence we recommend confirming the positive samples with a *Mycoplasma* agar plate or PCR method when it is applicable.

Performance Characteristics

1. Antimicrobials susceptibility coincidence rate

Inoculate 10^4-10^5 CCU/mL *UU* (ATCC® 33175) to the strip and incubate at 36-38℃ for 24h. The result of antimicrobials susceptibility is that the color of GAT (both low and high concentrations), ERY (both low and high concentrations), LVX^{UU}_{UP} (both low and high concentrations), MXF^{UU}_{UP} (both low and high concentrations) wells doesn't change; CLR, JOS, ROX, , wells at most one concentration turn red; PRI, OFL, MNO wells at least one concentration turn red; CLI (both low and high concentrations), TCY^{UU}_{UP} (both low and high concentrations) wells turn red.

Inoculate 10^4-10^5 CCU/mL *UP* (ATCC® 27813) to the strip and incubate at 36-38℃ for 24h. The result of antimicrobials susceptibility is that the color of PRI (both low and high concentrations), ERY (both low and high concentrations), MNO (both low and high concentrations), GAT (both low and high concentrations), LVX^{UU}_{UP} (both low and high concentrations), MXF^{UU}_{UP} (both low and high concentrations) well doesn't change; CLR, JOS, ROX, TCY^{UU}_{UP} wells at most one concentration turn red; OFL well at most one concentration turn red; CLI (both low and high concentrations) well turn red.

Inoculate 10^4-10^5 CCU/mL *MH* (ATCC® 23114) to the strip and incubate at 36-38℃ for 24h. The result of antimicrobials susceptibility is that the color of PRI (both low and high concentrations), OFL (both low and high concentrations), JOS (both low and high concentrations), MNO (both low and high concentrations), GAT (both low and high concentrations), LVX^{MH} (both low and high concentrations), TCY^{MH} (both low and high concentrations), MXF^{MH} (both low and high concentrations), CLI (both low and high concentrations) well doesn't change; ERY(both low and high concentrations), CLR (both low and high concentrations), ROX (both low and high concentrations) wells turn red.

It is acceptable that the color of low concentrations of ERY, LVX^{UU}_{UP}, MXF^{UU}_{UP}, LVX^{MH}, MXF^{MH}, CLI wells turn red and the color of high concentration don't change.

2. Specificity

Inoculate 10^4-10^5 CCU/mL *UU* (ATCC® 33175) to the MH well, MH≥10^4 well and UP well and incubate at 36-38℃ for 24 h. The result is valid if the color doesn't change.

Inoculate 10^4-10^5 CCU/mL *UP* (ATCC® 27813) to the UU well, MH≥10^4 well and MH well and incubate at 36-38℃ for 24h. The result is valid if the color doesn't change.

Inoculate 10^4-10^5 CCU/mL *MH* (ATCC® 23114) to the UU well, UU/UP≥10^4 well and UP well and incubate at 36-38℃ for 24h. The result is valid if the color doesn't change.

Inoculate the reference strain *E.coli* (ATCC® 25922), *C. albicans* (ATCC® 10231) and *Staphylococcus aureus* (ATCC® 29213) to the strip incubate at 36-38℃ for 24h. The result is valid if the color of the strip doesn't change.

3. Measurement Accuracy by Correlation

A study was performed where samples were tested using this assay and another CE marked assay and PCR method. For *UU/UP*, when the assay and PCR method generate a positive result, the *UU/UP* result is true positive; for *MH*, when the two assays generate a positive result, the *MH* result is true positive. Otherwise, the sample is negative. The PCR method is gold standard. The comparison between this assay and the reference assay and PCR method is presented below.
The result of *UP*:

		Reference assay and PCR method		
		Positive	Negative	Total
This assay	Positive	24	2	26
	Negative	1	149	150
Total		25	151	176

The result of *UU*:

		Reference assay and PCR method		
		Positive	Negative	Total
This assay	Positive	5	0	5
	Negative	0	171	171
Total		5	171	176

The result of *MH*:

		Reference assay and PCR method		
		Positive	Negative	Total
This assay	Positive	6	0	6
	Negative	0	170	170
Total		6	170	176

Literature References

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