

Only Myco Treatment WELL D-ONE®

System for the identification and antimicrobial susceptibility of urogenital mycoplasmas.

(These tests are in vitro diagnostic medical devices reserved for professional use)

1. INTRODUCTION

Mycoplasma hominis and *Ureaplasma urealyticum* / *parvum* belong to the Mollicutes classes and represents a group of complex microorganisms that are frequently isolated in the urogenital tract and can be considered opportunistic pathogens. A direct relationship has been shown between the isolation of these microorganisms and certain diseases such as bacterial vaginosis (BV), pelvic inflammatory disease, infections during pregnancy, infertility, preterm birth and neonatal infections ⁽¹⁻²⁻³⁻⁴⁾. In addition, some species of mycoplasma, known to be extremely fastidious, such as *Mycoplasma genitalium*, *Mycoplasma fermentans*, *Mycoplasma penetrans*, *Mycoplasma pirum* and others may colonize the genitourinary tract and cause non-gonococcal urethritis, sometimes asymptomatic, responsible for chronic processes, infertility, and tumors. ⁽⁵⁾ Some studies have shown that mycoplasmas, having the capacity to increase invasiveness of tumor cells ⁽⁶⁾, may be associated with oncogenesis and subsequent neoplastic diseases of the gastric, colon, lung, esophagus, breast ⁽⁷⁾ and prostate and uterine cancer ⁽⁸⁻⁹⁾. Rapid diagnosis available today may involve expensive tests not available in all hospitals. In light of this, a system that allows rapid growth in only 24-48 hours, without any additional equipment, can be an extremely useful tool in the hands of the microbiologist and the clinician.

2. PRINCIPLE

System consisting of a plastic plate composed by 16 conical wells for better visualization of colorimetric reactions that occur as a result of growth of microorganisms under examination in media specifically formulated for the selective culture of: *Mycoplasma hominis*, *Mycoplasma spp.*, *Ureaplasma urealyticum* / *parvum*.

3. CONTENT OF Only Myco Treatment WELL D-ONE®

(REF. MS01397)

10 test panels **Only Myco Treatment WELL D-ONE®**
10 x 10 mL Sterile Saline Solution
1 x 25 mL Sterile Liquid Paraffin
25 x 3 mL MYCO WELL D-ONE® TRANSPORT SCREEN

(REF. MS01398)

25 test panels **Only Myco Treatment WELL D-ONE®**
25 x 10 mL Sterile Saline Solution
1 x 40 mL Sterile Liquid Paraffin
10 x 3 mL MYCO WELL D-ONE® TRANSPORT SCREEN

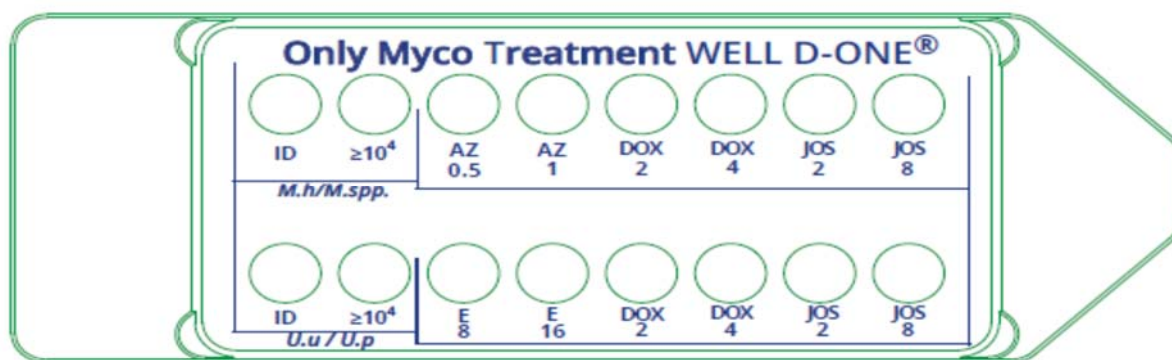
4. NECESSARY MATERIALS NOT INCLUDED

General laboratory equipment

5. STORAGE

Store at + 2°C/+8 °C in its original packaging. Do not store near heat sources and avoid extreme temperature variations. Under these conditions the product is valid until the expiration date indicated on the label of the primary and secondary container. Do not use after this date. Eliminate the products if there are signs of deterioration.

6. PANEL COMPOSITION



WELL	DESCRIPTION	CONTENT
1	ID <i>M. hominis</i>	Selective medium for the growth of <i>Mycoplasma hominis</i> and <i>M.spp</i>
2	$\geq 10^4$ <i>M. hominis</i>	Selective medium for the growth of CCU $\geq 10^4$ <i>Mycoplasma hominis</i>
3	AZ 0.5 µg/mL	Culture medium containing Azithromycin 0.5 µg/mL
4	AZ 1 µg/mL	Culture medium containing Azithromycin 1 µg/mL
5	DOX 2 µg/mL	Culture medium containing Doxycycline 2 µg/mL
6	DOX 4 µg/mL	Culture medium containing Doxycycline 4 µg/mL
7	JOS 2 µg/mL	Culture medium containing Josamycin 2 µg/mL
8	JOS 8 µg/mL	Culture medium containing Josamycin 8 µg/mL
9	ID <i>U.urealyticum/parvum</i>	Selective medium for the growth of <i>Ureaplasma urealyticum/parvum</i>
10	$\geq 10^4$ <i>U.urealyticum/parvum</i>	Selective medium for the growth of CCU $\geq 10^4$ <i>Ureaplasma urealyticum/parvum</i>
11	E 8 µg/mL	Culture medium containing Erythromycin 8µg/mL
12	E 16 µg/mL	Culture medium containing Erythromycin 16 µg/mL
13	DOX 2 µg/mL	Culture medium containing Doxycycline 2 µg/mL
14	DOX 4 µg/mL	Culture medium containing Doxycycline 4 µg/mL
15	JOS 2 µg/mL	Culture medium containing Josamycin 2 µg/mL
16	JOS 8 µg/mL	Culture medium containing Josamycin 8 µg/mL

7. TEST PROCEDURE

7.1 SAMPLE COLLECTION AND PREPARATION

Samples: endocervical exudate, vaginal exudate, urethral exudate, urine, seminal fluid, endotracheal aspirate / exudate. To obtain the best performance with the use of this system, the sample should be collected aseptically according to the methodology implemented in each hospital center and, in any case, before starting the antibiotic treatment.

a. If the sample is not processed immediately after collection:

1) Endocervical exudate, vaginal exudate, urethral exudate: Collect the specimen with a sterile	1) Endotracheal suction / exudate tract samples: Collect the specimen with a sterile	1) Liquid samples, urine, seminal fluid: Take 200 µL of sample
2) Introduce the swab (or 200µL of liquid sample) into the transport medium MYCO WELL D-ONE® SAMPLE COLLECTION KIT after bringing it back to room temperature. <ul style="list-style-type: none"> - resuspend, press against the wall of the bottle to obtain a homogeneous solution. - correctly close the bottle, enter the necessary information for the identification and traceability of the sample. - throw the swab. <p>* MYCO WELL D-ONE® SAMPLE COLLECTION KIT medium allows the transport and conservation of samples for a period of 48 hours at room temperature (+18°C/+25°C), 3 days at + 4°C, or for a period of 8 weeks at -20°C/-70°C.</p>		

3) To inoculate the sample on the plate Only Myco Treatment WELL D-ONE® :

- Add 4 drops (200 µL) of MYCO WELL D-ONE® SAMPLE COLLECTION KIT inoculated into the saline solution provided in the kit
- Add 150 µL of the suspension obtained in each well of the Only Myco Treatment WELL D-ONE® plate.
- **/!** Add two drops of sterile paraffin to all wells of the plate

b. If the sample is processed immediately after collection:

In the case of endocervical exudate, vaginal exudate, urethral exudate, endotracheal exudate:

- Take the sample according to the procedures established in the laboratory.
- Resuspend the sample in the sterile saline solution provided in the kit.
- Leave in the saline solution for 3-5 minutes.
- Shake the swab and press against the walls until a homogeneous suspension is obtained.

OR

- In the case of urine, seminal fluid:
- Resuspend 200 µL of the sample in the saline solution.

THEN

- Add 3 drops (150 µL) of the suspension obtained in each well of the plate.
- **/!** Add two drops of sterile paraffin to all wells of the plate.
- Incubate at $+36 \pm 1^\circ\text{C}$ for **24-48H**, up to 7 days to identify *Mycoplasma spp.*

7.2 Presumptive identification of the microorganism included in the system

• **IDENTIFICATION AND TITRATION OF *Mycoplasma hominis***

The *Mycoplasma hominis* section can become positive after 24 to 48 hours of incubation at $+36 \pm 1^\circ\text{C}$. The identification of *Mycoplasma hominis* at pathogenic thresholds is given by:

- ✓ shift of color from transparent yellow to transparent RED of WELL 1 (ID) (and 2 if $\geq 10^4$ CCU/mL). Change of color from transparent orange to transparent RED in 24/48 Hours means *M. hominis* is found in the sample.

/! To guarantee the conformity of the results and the specificity of the colorimetric reactions, all the wells of the plate must stay transparent. The presence of turbidity is evidence of contamination.

/! A non-specific change of color without turbidity may occur due to some bacterial strains that do not belong to the genus *Mycoplasma*, or certain yeasts

• **IDENTIFICATION OF *M.spp* AND CONTROL WELL for *Mycoplasma hominis*:**

Well 1 (ID) contains a culture medium that allows the growth of the different species of *Mycoplasmas spp.* existing in the sample. The original color of the well is transparent ORANGE.







Strains belonging to the genus *Mycoplasma* different from *M. hominis*, such as *M. genitalium*, *M. pirum*, *M. penetrans*, *M. fermentans*, can grow in Well 1 (ID). Their growth causes a change of color from transparent orange to transparent YELLOW.

- ✓ A progressive change of color from transparent orange to transparent YELLOW after 72 hours means other glycolytic *M.spp* species are found in the sample. This positive result must be confirmed by additional detection techniques for mycoplasma identification (PCR). In order to carry out this confirmation test, you must carefully take the content of the well.
- ✓ In the rare event that *M. hominis* and other species are found in the sample, the well may show a change to transparent red first and then a slow change to transparent orange after 72 hours. However, additional tests are recommended to prove it.

/! Color change from transparent orange to transparent YELLOW in Well 1 (ID), before 48 hours is not a growth index of *Mycoplasma spp.* In this case, it is recommended to carry out confirmatory tests such as traditional culture or molecular methods.
(15-16)





/! Well 1 (ID) is able to show *M.spp*, after 3 days of incubation at $+36 \pm 1^\circ\text{C}$. Up to 7 days of incubation may be necessary. In case of a prolonged incubation of the plate for the detection of *M.spp*, only the colorimetric changes of the M.h/M.spp SECTOR should be observed. DO NOT consider the Uu/Up SECTOR.

• **READINGS OF THE WELLS AT 24-48H FOR *M.hominis***

Results in 24-48H		
Example 1: Well 1 is red. Well 1 (ID) is RED.	 ID  $\geq 10^4$	Positive for <i>M.h</i>
Example 2: Well 1 (ID) and 2 are RED.	 ID  $\geq 10^4$	Positive for <i>M.h</i> $\geq 10^4$ CCU /mL
Example 4: Well 1 (ID) and 2 are ORANGE.	 ID  $\geq 10^4$	Negative for <i>M.h.</i> , wait at least 72 hours to obtain <i>M.ssp</i> results.

The time needed to show positivity in the wells depends on the strain and the concentration in which it is found in the sample.

• **READINGS OF THE WELLS AT 72H FOR *M.spp***

Results after 72H		
Example 1: 1 (ID) is YELLOW.	 ID  $\geq 10^4$	Positive for <i>M.spp</i> .
Example 2: 1 (ID) is ORANGE	 ID  $\geq 10^4$	Negative for <i>M.spp</i> .

***!/** In the rare event that *M. hominis* and other species of *M.spp.* are found in the sample, the well may show a change to transparent RED first and then a slow change to transparent ORANGE after 72 hours. However, additional tests are recommended to prove it.

• **IDENTIFICATION AND TITRATION OF *Ureaplasma urealyticum/parvum***





The *Ureaplasma spp.* section can become positive after 18 to 24 hours of incubation at $+36 \pm 1^\circ\text{C}$. Some “fastidious” strains of *Ureaplasma spp.* may require up to 36 hours to grow. The identification of *U.urealyticum/parvum* at pathogenic thresholds is given by:

- ✓ shift of color from transparent yellow to transparent RED of WELL 9 (and 10 if $\geq 10^4$ CCU/mL)

!/ To guarantee the conformity of the results and the specificity of the colorimetric reactions, all the wells of the plate must stay transparent. The presence of turbidity is evidence of contamination.

!/ A non-specific change of color without turbidity may occur due to some bacterial strains that do not belong to the genus *Ureaplasma*, or certain yeasts.

• **READINGS OF THE WELLS FOR *Ureaplasma spp.* (*U.urealyticum* et/ou *U.parvum*)**

Example 1: Well 9 is RED.	 ID  $\geq 10^4$	Positive for <i>Ureaplasma spp.</i>
Example 2: Well 9 and 10 are RED.	 ID  $\geq 10^4$	Positive for <i>Ureaplasma spp.</i> , $\geq 10^4$ CCU /mL.

The time needed to show positivity in the wells depends on the strain and the concentration in which it is found in the sample.

8. INTERPRETATION OF RESULTS

- Observe color variations in the wells based on chemical and / or chromogenic components reactions contained in specific formulations developed for each microorganism (see annexed table).
- Microscopic observation is recommended as shown in this leaflet.

9.RECOMMENDATIONS AND PRECAUTIONS

- 1.Only for professional diagnostic *in vitro* use.
- 2.Samples should be treated as potentially infectious and tests should only be performed by qualified and trained personnel to perform clinical and / or microbiological laboratory techniques.
3. Do not use if signs of deterioration of the kit components are observed.
4. Incubations longer than 24 to 48 hours may be performed on the basis of a microbiologist's evaluation for a period of 7 days, but the formulations of this kit allow the growth of the most common urogenital mycoplasmas (*Mycoplasma hominis* and *Ureaplasma urealyticum / parvum*) in 18 -48 hours ⁽¹⁹⁾.

5. The tests should be discarded in a proper biohazard container after testing.

10. LIMITATIONS:

- Samples obtained and collected during antibiotic treatment may give negative results ⁽¹⁴⁻¹⁸⁾.
- Samples such as vaginal or endocervical exudates with unusual pH values due to particular inflammatory processes, or inadequate collection and / or storage of the sample, may interfere with the correct interpretation and functionality of this system, non-specific reactions in identification wells may occur ⁽¹⁵⁻¹⁶⁾. Carefully observe the colors of all wells to identify these interferences.
- A sample with a high concentration of CCU of urogenital mycoplasmas may result in a generalized positivity in the identification panel. In this case, it is recommended to dilute the sample (1: 2) ⁽¹⁰⁻¹⁷⁾. Take into account the dilution for the interpretation.
- Inadequate collection or storage of the sample may result in microbiological contamination or agent's incompatible with the proper functioning of this system.
- The presence of Urea-producing enzyme microorganisms gives a positive reaction in well number 6. Read these Instructions carefully before carrying out the test in order to avoid errors.

11. QUALITY CONTROL

The reference strains are used for both the positive reactions of the different wells as to test the good functioning of the media formulations of the different wells in the event of any unspecific reactions.

To perform quality control, it is recommended to use the following reference strains:

Strains	ATCC
<i>Mycoplasma hominis</i>	23114
<i>Ureaplasma urealyticum</i>	27618
<i>Ureaplasma urealyticum</i>	33175
<i>Ureaplasma parvum</i>	27815
<i>Mycoplasma genitalium</i>	33530
<i>Mycoplasma fermentans</i>	19989
<i>Mycoplasma hyorhinis*</i>	29052

The strains used for quality control were selected on the basis of published for growth and susceptibility test recommendations ⁽¹²⁻¹⁸⁾. Each laboratory must establish its internal quality controls.

12. FUNCTIONAL CHARACTERISTICS ⁽¹⁹⁾

The performance of the products of MYCO WELL D-ONE® line was carried out by growth and antibiotic susceptibility test with broth microdilution method of ATCC reference strains and recommended reference strains for the quality control procedure of urogenital mycoplasma. A concordance of 100% has been observed.

The media used for the growth, identification and susceptibility of *Mycoplasma hominis*, *Ureaplasma urealyticum* / *parvum* and the identification of *Mycoplasma spp.*, were tested in comparison with traditional culture methods and molecular methods using samples from different collection sites (vaginal exudate, urethral exudate, urine, respiratory samples) with >99% specificity and sensitivity for identification, isolation and susceptibility of different species of mycoplasmas and microorganisms responsible for infections of the genitourinary tract of clinical samples.

TABLE OF COLORIMETRIC REACTIONS

COLORIMETRIC REACTION FOR MICROBIAL IDENTIFICATION			
<i>Mycoplasma hominis</i>			
WELL	IDENTIFICATION	POSITIVE	NEGATIVE
1 (For <i>M.hominis</i> and <i>M.spp</i>)	<i>M. hominis</i>	RED (24-48H)	YELLOW
	<i>M.spp</i>	YELLOW (>72H)	RED
2 (ONLY for <i>M.hominis</i>)	$\geq 10^5$ <i>M. hominis</i>	RED	YELLOW
<i>U.urealyticum/parvum</i>			
WELL	IDENTIFICATION	POSITIVE	NEGATIVE
9	10^4 <i>U.urealyticum/parvum</i>	RED	YELLOW
10	$\geq 10^5$ <i>U.urealyticum/parvum</i>	RED	YELLOW











The SUSCEPTIBILITY SECTOR is divided for <i>M.hominis</i> and <i>U.urealyticum/parvum</i>		
WELL	RESISTANT	SENSITIVE
3 to 8 (<i>M.hominis</i>)	RED	ORANGE
3 to 8 (<i>M.spp</i>)	YELLOW	ORANGE
11 to 16 <i>U.urealyticum/parvum</i>	RED	YELLOW

14. BIBLIOGRAPHY

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19. Validation des systèmes WELL D-ONE, Technical Files, C.P.M. SAS, 2013-2017

Symbols Table

	Codice	Reference number	Código
	Lotto	Batch number	Número de lote
	Scadenza	Expiration date	Fecha de vencimiento
	Temperatura	Temperature	Temperatura
	Per uso diagnostico <i>in vitro</i>	For <i>in vitro</i> diagnostic use	Para uso de diagnóstico <i>in vitro</i>
	Fragile, manipolare con cura	Fraille, handle with care	Frágil, manejar con cuidado
	Istruzioni per l'uso	Instruction for use	Literatura Interior
	Fabbricante	Manufacturer	Fabricante
	Contenuto sufficiente per <n> test	Content sufficient for <n> tests	Contenido suficiente para <n> pruebas
	Non riutilizzare	Do not re-use	No reutilizar



Conforme alla direttiva 98/79 CE dei dispositivi medico diagnostici *in vitro*
 Conform to the Directive 98/79/EC on *in vitro* diagnostic medical device
 Conforme a la direttiva 98/79/CE de dispositivos médicos para diagnóstico *in vitro*



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