MYCO WELL D-ONE ® *SCREEN*

System or the identification of uogenital mycoplasmas, *Streptococcus B, Candida spp., Gardnerella vaginalis* and *Trichomonas vaginalis*

1 . INTRODUCTION

Mycoplasma hominis ed *Ureaplasma urealyticum / parvum* belong to the Mollicutes class and represent 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.

In some cases, the positivity of urogenital mycoplasmas is associated with the presence of other microorganisms that cause genitourinary infections, such as *Streptococcus agalactiae* (Group B), *Gardnerella vaginalis, Trichomonas vaginalis* and *Candida albicans*. Simultaneous identification of these agents can provide a valid orientation on clinical diagnosis and on the procedure to be followed with the patient.[1][10][11]

2 . PRINCIPLE

System consisting of a plate containing 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*.

In addition, the kit allows the identification of *Streptococcus agalactiae* (Group B), *Gardnerella vaginalis, Trichomonas vaginalis* and *Candida spp.*

The plate contains control wells for detecting of cross-reactions and / or non-specific reactions caused by the sample type, inadequate manipulations, contamination.

In case of a positive result, the investigation can be thoroughly examinated using the MYCO WELL D-ONE® AST system for antimicrobial susceptibility tests in accordance with the CLSI guidelines [12].

3. CONTENT OF MYCO WELL D-ONE® SCREEN (REF.MS01346)

25 test panels MYCO WELL D-ONE® SCREEN
25 x 10 mL Sterile Saline solution
1 x 35 mL Liquid Sterile Paraffin

4. NECESSARY MATERIALS NOT INCLUDED

MYCO WELL D-ONE® *SAMPLE COLLECTION KIT*
(REF. MS01348 containing 25 Sterile Swabs and 25 MYCO WELL D-ONE® *TRANSPORT SCREEN*)
General laboratory equipment

5. PANEL COMPOSITION

WELL	DESCRIPTION	CONTENT
1	ID *M. hominis*	Selective medium for the growth of *Mycoplasma hominis*
2	10^4 *M. hominis*	Selective medium for the growth of CCU 10^4 *Mycoplasma hominis*
3	≥10^5 *M. hominis*	Selective medium for the growth of CCU ≥10^5 *Mycoplasma hominis*
4	ID *U.urealyticum/parvum*	Selective medium for the growth of *Ureaplasma urealytium/parvum*
5	10^4 *U.urealyticum/parvum*	Selective medium for the growth of CCU 10^4 *Ureaplasma urealytium/parvum*
6	≥10^5 *U.urealyticum/parvum*	Selective medium for the growth of CCU ≥10^5 *Ureaplasma urealytium/parvum*
7	Urease +	Culture medium for the identification of microorganisms producing urea, not belonging to the ureaplasma species
8	Cross Reaction	Terreno di coltura per la determinazione di reazioni crociate nei pozzetti da 1 a 7
9	ID *M. spp*	Selective medium for the growth of *Mycoplasma spp.*
10	ID *M. spp*	Selective medium for the growth of *Mycoplasma spp.*
11	ID *M. spp*	Selective medium for the growth of *Mycoplasma spp.*
12	Cross Reaction	Culture medium for the determination of cross-reactions of wells 9-10-11
13	StB	Chromogenic selective medium for the identification of *Streptococcus agalactiae*
14	CAN	Chromogenic medium for the identification of di *Candida spp.*
15	Gv	Selective medium for the identification of*Gardnerella vaginalis*
16	Tv	Selecetive medium for the identification of *Trichomonas vaginalis*

6. TEST PROCEDURE

6.1 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 at each hospital center and, in any case, before starting the antibiotic treatment.

6.2 Procedure (endocervical exudate, vaginal exudate, urethral exudate)

1. Collect the sample with a sterile swab.
2. Open the vial containing the transport medium MYCO D-ONE TRANSPORT *SCREEN.*
3. The transport medium MYCO D-ONE TRANSPORT *SCREEN*, allow the transport and conservation of the samples for 24 hours at room temperature (18-25 °C), 3 days at +4 °C or 8 weeks at – 20 °C / 70 °C.
4. Introduce the swab, resuspend it properly, close the vial and discard the swab.
5. To inoculate the sample in the panel identification, collect 500 µL/10 drops of inoculated MYCO D-ONE TRANSPORT *SCREEN* and add it in the saline solution provided with the kit and wait 5 minutes.
6. Add 150 µL (3 drops) of the obtained solution in each well of the MYCO WELL D-ONE® *SCREEN* plate.
7. Add 2 drops of liquid sterile paraffin on the wells from 1 to 13
1. Incubate at 36±1 °C for 24-48 hours, 3-7 days for the investigation of *Mycoplasma spp.*
8. All readings are realized visually.
9. In the case of well 25-CAN and 16-Tv it is recommended a complementary microscopic observation (40 X).

6.3 Procedure to perform in case of other samples (urine, seminal fluid, endotracheal aspirate / exudate)

1. Samples of the respiratory tract or associated: take a sample aliquot with a sterile swab and perform the same procedure as described before paragraph 6.2
2. In case of liquid samples (urine, seminal fluid), collect 200 µL of sample and add it the transport medium MYCO D-ONE TRANSPORT *SCREEN.*

3. The transport medium MYCO D-ONE TRANSPORT *SCREEN*, allow the transport and conservation of the samples for 24 hours at room temperature (18-25 °C), 3 days at +4 °C or 8 weeks at – 20 °C / 70 °C.
4. To inoculate the sample in the panel identification, collect 500 µL/10 drops of inoculated MYCO D-ONE TRANSPORT *SCREEN* and add it in the saline solution provide with the kit and wait 5 minutes.
5. Add 150 µL (3 drops) of the obtained solution in each well of the MYCO WELL D-ONE® *SCREEN* plate.
6. Add 2 drops of liquid sterile paraffin on the wells from 1 to 13
7. Incubate at 36±1 °C for 24-48 hours, 3-7 days for the investigation of *Mycoplasma spp.*
8. All readings are realized visually.
9. In the case of well 25-CAN and 16-Tv wells it is recommended a complementary microscopic observation (40 X).

6.4 Incubation

It is recommended to incubate the plate for 24/48 hours and up to 7 days for identification of *Mycoplasma spp.*

6.5 Procedure to be followed in case of immediate test after collection

If the sample is processed immediately after collection, proceed as follows:

Endocervical exudate, vaginal exudate, urethral exudate, urine, seminal fluid, endotracheal aspirate / exudate

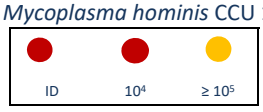
1. Collect the sample according to the procedures established in each laboratory.
2. Resuspend the swab in the sterile physiological solution supplied in the kit, leave in the saline for 3-5 minutes, shake the swab and press against the vial walls until a homogeneous suspension is obtained.
3. In case of Urine-Seminal Liquid, resuspend 200 µL of the sample in the saline solution.
4. Dispense 150 µL of the final suspension obtained in each well of the system panel.
5. Add two drops of sterile paraffin into the wells 1 to 13
6. Incubate at 36 ± 1 °C for 24-48 hours, 3-7 days for the investigation of Mycoplasma spp.
7. All readings are realized visually.
8. In the case of the 25-CAN and 16-Tv wells it is recommended a complementary microscopic observation (40 X).

6.6 Presumptive identification of the microorganisms included in the System

- **IDENTIFICATION OF *Mycoplasma hominis***

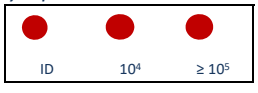
Mycoplasma hominis is positive after 24-48 hours. The identification of *Mycoplasma hominis* is given by the positivity of wells 1, 2 and 3. The well 3 is positive only if the concentration of CCU is ≥ 10^5.

Mycoplasma hominis CCU 10^4



Mycoplasma hominis

Mycoplasma hominis CCU ≥ 10^5



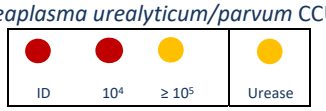
Mycoplasma hominis

- **IDENTIFICATION OF *Ureaplasma urealyticum/parvum***

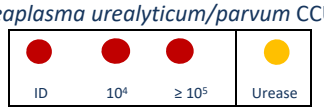
Ureaplasma spp. is positive after 24 hours. Some "fastidious" strains of di *Ureaplasma spp.* Can be positive after 36 hours. The identification of *Ureaplasma urealyticum/parvum* is given by the positivity of wells 4, 5 e 6. The well 6 is positive only if the CCU of *Ureaplasma urealyticum/parvum* is ≥10^5.

The strains belonging to *Ureaplasma spp*. qre not able to growth in well 7.

Ureaplasma urealyticum/parvum CCU 10^4



Ureaplasma urealyticum/parvum CCU≥ 10^5



• **<u>IDENTIFICATION OF *M.spp*</u>**

Mycoplasma spp., can be positive after 3 days of incubation. The incubation can be prolonged until 7 days for the "fastidious" species. An incubation longer than 7 days can be realized at the discrection of microbiologist.

Some *M.spp* strains, turn into yellow color in the identification wells of the *M. spp* in different periods of time. The positivity of at least one well in the *M.spp* sector after 3 days of incubation it is a sign of positivity. The color change is slow and progressive over time.

Notes and recommendations:

• The presence of a transparent yellow color in the ID well for *M. spp*, must be confirmed by additional detection techniques for mycoplasmas identification. In order to carry out this confirmation test it must carefully take the contents of the well.

• In the case of wells 9-10-11 (*Mycoplasma spp*) a color variation, index of fermentative mycoplasmas species is not observed until 3-7 days later, according to the species. In the case of prolonged incubation of this type, only the wells 9-10-11 should be observed and the colorimetric variation of the rest of the wells of the identification plate must not be considered.

• Growth in wells 9-10-11 is shown by change in color from red to transparent-yellow. Turbid color or presence of sediment is the index of the growth of a microorganism in the sample, not belonging to the *Mycoplasma* species.

• Color change in wells 9-10-11, before 48 hours is not a growth index of *Mycoplasma* species. In this case, it is recommended to carry out confirmatory tests such as traditional culture or molecular methods. [15-16]

• Because samples such as vaginal or endocervical exudates with infrequent pH values due to particular inflammatory processes may cause non-specific reactions with positive coloration in the identification wells for urogenital mycoplasmas [15-16] or inadequate sample collection or storage may result in microbial contamination that may interfere with the correct interpretation and functionality of this system, Cross Reactions wells 8 and 12 allow to specifically identify cross-reaction and non-specific reactions in order to avoid interpretative errors. In case of the positivity of the wells described above, evident turbidity will be observed.

• It is recommended to carry out the latex agglutination test on the contents of the well 13 *Streptococcus agalactiae*. Only a few non-hemolytic *S. agalactiae* strains may take up to 48 hours to vary the color of the well, or cause turbidity without colorimetric reaction. The realization of the latex test of the contents of the well accelerates microbial identification.

• In the case of the 14-CAN well, the presence of *Candida albicans* is indicated by a color change to Green. In case of other *Candida* species, other types of coloration will be observed (as indicated in the colorimetric table attached to

these Instructions for Use). Microscopic observation (40 X) of 14-CAN well is it recommended for chlamydospore/hyphae observation.

- In the case of 15-Gv well, a red color indicates a bacterial growth. A microscopic obeservation (100X) it is recommended to observe the morphological characteristics of *Gardnerella vaginalis*.
- In the case of 16-Tv well, no color change will be observed and it is recommended a microscopic observation non si (40 X) of the culture medium contained in the well. Observe the morphological characteristis of *Trichomonas vaginalis*.
- 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].
- In the case of incubations longer than 48 hours, 14-CAN, 15-Gv and 16-TV well volume content decrease may be detected, indicated for microscopic observation. In this case, colorimetric reading is not compromised; nevertheless, in order to perform microscopic observation, two drops of saline solution may be added to resuspend the contents before preparing the slide for microscopic observation. The presence of color in the wells does not interfere with the observation of the microbial structure.
- It is recommended to use conventional methods for identification of microorganisms that can be identified by this test [18].

7. 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.
It is recommended to use MYCO WELL D-ONE® AST in the case of positive samples that must be confirmed and subjected to antibiotic susceptibility testing.

8.WARNING AND PRECAUTIONS

1.Only for professional diagnostic *in vitro* use
2.Samples should be treated as potentially infectious and tests should only be effected 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.

9.LIMITATIONS

Samples obtained and collected during antibiotic treatment may give negative results [14-18].
Samples such as vaginal or endocervical exudates, with infrequent pH values due to particular inflammatory processes, may cause non-specific reactions with positive coloration in the identification wells for urogenital micoplasms [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 these cases it is recommended to dilute the sample [10-17].
Inadequate collection or storage of the sample may result in microbiological contamination or agents incompatible with the proper functioning of this system.
The presence of Urea-producing enzyme microorganisms give a positive reaction in the 7-Urease + well.

Read these Instructions carefully before carrying out the test in order to avoid errors.

10.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:

"Non fastidious" mycoplasmas

Strain	ATCC
Mycoplasma hominis	23114
Ureaplasma urealyticum	27618
Ureaplasma parvum	27815

"fastidious" or slow growth mycoplasmas

Ceppo	ATCC
Mycoplasma genitalium	33530
Mycoplasma fermentans	19989
Mycoplasma penetrans	55252
Mycoplasma pirum	25960
Mycoplasma hyorhinis*	29052

*The inclusion of the *Mycoplasma hyorhinis* strain (cultivar alpha) was included to demonstrate the ability of the formulation to promote the growth of fastidisous species (*M. genitalium* and *M. fermentans*) in traditional culture media because growth dynamics and nutritional requirements of these species are very similar.

Other tested strains:
Candida albicans ATCC 10231
Candida tropicalis ATCC 13803
Candida krusei ATCC 14243
Gardnerella vaginalis ATCC 14019
Trichomonas vaginalis ATCC 30001
Streptococcus agalactiae ATCC 13813

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.

11. 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., Streptococcus agalactiae (group B), Gardnerella vaginalis, Trichomonas vaginalis, Candida albicans* and *Candida 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 about 100% 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.

13. STORAGE

Store at 2-8 °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 if there are signs of deterioration.

14. BIBLIOGRAPHY

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

TABLE OF COLORIMETRIC REACTIONS

		Colorimetric reaction	
WELL	**DESCRIPTION**	**POSITIVE**	**NEGATIVE**
1	ID *M. hominis*	RED	YELLOW
2	10^4 *M. hominis*	RED	YELLOW
3	$\geq 10^5$ *M. hominis*	RED	YELLOW
4	ID *U.urealyticum/parvum*	RED	YELLOW
5	10^4 *U.urealyticum/parvum*	RED	YELLOW
6	$\geq 10^5$ *U.urealyticum/parvum*	RED	YELLOW
7	Urease +	RED	YELLOW
8	Cross Reaction	TURBIDITY	TRANSPARENT WHITE
9	ID *M. spp*	YELLOW	RED
10	ID *M. spp*	YELLOW	RED
11	ID *M. spp*	YELLOW	RED
12	Cross Reaction	TURBIDITY	TRANSPARENT WHITE
13	StB	GREEN	SLIGHT YELLOW
14	CAN	GREEN*[1]	NO COLOR/TURBID WHITE
15	Gv	RED	NO COLOR
16	Tv	MICROSCOPIC OBSERVATION 40X	MICROSCOPIC OBSERVATION 40X

*[1] A color change to GREEN is indicative of the presence of *Candida albicans*
In the presence of *Candida tropicalis* the well will assume a VIOLET color
Other *Candida* species may give other colorations in the well

Symbols Table

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

CE

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

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