MYCO PNEUMO ONE®

System for the presumptive identification and antibiotic susceptibility test of *Mycoplasma pneumoniae*

1.INTRODUCTION

Mycoplasma pneumoniae causes up to 10-40% of community-acquired pneumonia. The incidence of *M. pneumoniae* pneumonia is higher among children and young adults.
The symptoms of *M. pneumoniae* infections of the upper and lower respiratory tract are generally mild and often self-limiting. The most frequent extrapulmonary complications are associated with the CNS, the heart and the skin. Skin diseases usually occur with erythematous or transient maculopapular blisters, but can sometimes develop into Stevens-Johnson syndrome.
A percentage of asthmatic exacerbations is related to *M. pneumoniae*, as a consequence of chronic diseases of the lower respiratory tract. Numerous studies have also shown a link between *M.pneumoniae* and recurrent asthmatic processes or that do not respond to conventional treatments.
The presence of these microaganisms has also been associated with ocular infections [(1-8)].
As a result, the logical procedure would be to eliminate these pathogens as soon as possible using antibiotics. However, antibiotics are not recommended for the treatment of acute asthma exacerbations, except when necessary. These differences underscore the need to define the role of antimicrobials that are active against atypical primarily macrolide pathogens, but also tetracyclines and fluoroquinolones in the treatment of asthma[(5-7)].
A system that allows accelerated growth, with antimicrobial susceptibility in just 48 hours, without additional equipment, can be a useful tool in the hands of the microbiologist and the clinician in the diagnosis of *Mycoplasma pneumoniae*.

2. PRINCIPLE

System consisting of a plate containing 16 conical wells designed for better visualization of colorimetric reactions that occurs as a result of growth and antimicrobial susceptibility testing in culture media especially formulated for selective culture and AST of *Mycoplasma pneumonia.*
The plate also contains control wells for the detection of cross-reactions and / or non-specific reactions caused by the type of sample, inadequate manipulation, contamination.
The system is inoculated with the test sample and incubated at 36 ± 1 ° C for 48 hours. Following incubation, the micro-organism (or the eventual copresence of several micro-organisms) is identified by combining the colorimetric reactions that take place in the wells.
Antimicrobial susceptibility is designed to help in the identification and evaluation of cut off susceptibility and resistance of antibiotics as recommended by CLSI.
Microbiological diagnoses can be confirmed by serological tests, microscopic observation, culture or molecular techniques directly from the positive wells.

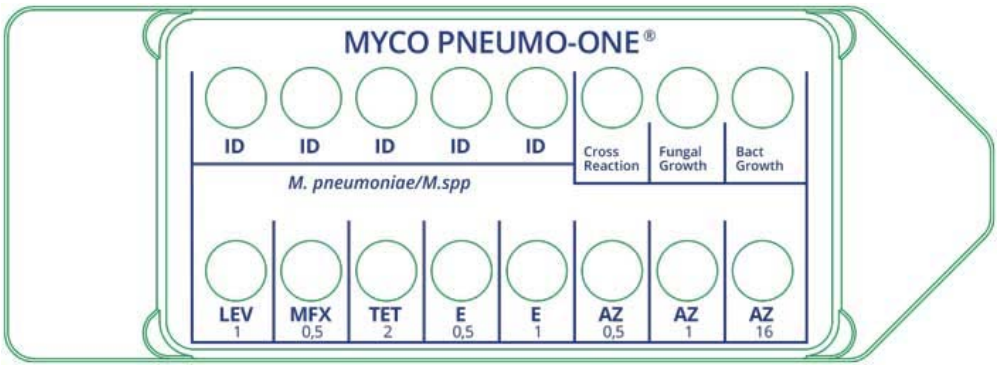
3. CONTENT OF MYCO PNEUMO ONE KIT REF. MS01352

10 Identification panels
10 Storage Liquide Solutions *Mycoplasma spp.*

4.REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

Culture Medium Plates enriched for *Mycoplasma spp.*
Reagents for Gram Stain
Sterile liquid paraffin
Sterile swabs
Generic laboratory material

5. PANEL IDENTIFICATION DESCRIPTION



WELL	DESCRIPTION	CONTENT
1	*M.pneumoniae/M.spp*	Selective medium for the presumptive identification of *Mycoplasma spp.*
2	*M.pneumoniae/M.spp*	Selective medium for the presumptive identification of *Mycoplasma spp.*
3	*M.pneumoniae/M.spp*	Selective medium for the presumptive identification of *Mycoplasma spp..*
4	*M.pneumoniae/M.spp*	Selective medium for the presumptive identification of *Mycoplasma spp..*
5	*M.pneumoniae/M.spp*	Selective medium for the presumptive identification of *Mycoplasma spp..*
6	Cross Reaction	Selective medium for the determination of cross-reactions.
7	Fungal Growth	Selective medium for the identification of fungi and yeasts in the sample
8	Bact Growth	Selective medium for the identification of bacteria not belonging to the *Mycoplasma* species.
9	LEV 1	Selective medium for *Mycoplasma spp.* containing Levofloxacin 1 µg/mL
10	MXF 0,5	Selective medium for *Mycoplasma spp.* containing Moxifloxacin 0,5 µg/mL
11	TET 2	Selective medium for *Mycoplasma spp.* containing Tetaraciclin 2 µg/mL
12	E 0,5	Selective medium for *Mycoplasma spp.* containing Erythromycin 0,5 µg/mL
13	E 1	Selective medium for *Mycoplasma spp.* containing Erythromycin 1 µg/mL
14	AZ 0,5	Selective medium for *Mycoplasma spp.* containing Azitromicin 0,5 µg/mL
15	AZ 1	Selective medium for *Mycoplasma spp.* containing Azitromicin 1 µg/mL
16	AZ 16	Selective medium for *Mycoplasma spp.* containing Azitromicin 16 µg/mL

6. EXECUTION OF THE TEST

6.1 SAMPLES AND PROCEDURE

SAMPLES:
Pharyngeal swab, nasopharyngeal swab, bronchial secretions, tracheal aspiration, pleural fluid aspiration, transthoracic needle aspiration, trans-tracheal needle aspiration, broncho-alveolar lavage, pulmonary needle aspiration, abscess needle aspiration, blood cultures.

To obtain the best performances with the use of this system, the sample must be collected aseptically according to the methodology implemented in each hospital center and before starting the antibiotic treatment.
The sample must be tested immediately after collection (Paragraph 6.3).
Do not refrigerate or freeze. Protect from light and sun.
The **MYCO PNEUMO ONE ®** system must be used by a qualified microbiologist.
In case of doubt in the procedure or in the interpretations, contact the product specialist of C.P.M. on site or directly at the central office.
Whenever possible, it is recommended that respiratory tract samples are grown in conventional media such as Columbia Agar with 5% sheep blood.

6.2 PROCEDURE FOR TEST EXECUTION

In the case of pharyngeal and nasopharyngeal exudates, resuspend the swab containing the sample in the vial of the Storage Liquide Solutions *Mycoplasma spp.* provided in the kit, shake the swab and press it against the walls to obtain a homogeneous suspension. In the case of other types of respiratory samples to be analyzed, using a Pasteur pipette or a sterile swab, resuspend a portion of the sample directly in the Storage Liquide Solutions *Mycoplasma spp*.
Of the suspension obtained, inoculate 150 µL in each well of the **MYCO PNEUMO ONE** panel.
WARNING the samples must be collected according to the sample collection procedure established in each laboratory and based on the standard guidelines [17-20] (see section 6.3).

/! Add two drops of sterile paraffin to wells 1 to 16.

/! The other samples of the tract respiratory to be analyzed using the **MYCO PNEUMO ONE** system must be processed as established in each laboratory, according to what is reported in section 6.3.
The microbiologist must evaluate what type of sample should be inoculated directly into the Storage Liquide Solution *Mycoplasma spp.*

Incubation

Incubate at 36 ± 1 ° C for 48 hours, in a traditional laboratory incubator.
The incubation can be extended up to 5 days, taking into account the time required for the growth of the microorganisms included in the identification panel and their concentration in the sample in examination.

6.3 RECOMMENDATIONS FOR THE COLLECTION AND SAMPLE ANALYSIS

Each hospital and / or laboratory center must follow the procedures established internally for the selection of the samples to be studied. The recommendations presented here are for guidance only .

Sample collection, aspects to consider: [1] [4] [5]

- Assess the risk / benefit of patient sample collection, especially in case of invasive samples.
- Samples must be transported in suitable containers.
- Sample collection must be carried out under aseptic conditions
- An adequate amount of sample must be collected.

if it was necessary to transport or store the sample until its analysis, it is recommended to use an adequate transport medium. Use of transport media such as Amies or Stuart interfere with the results of this kit.
The sample must be analyzed before two hours from collection, and conserved into the Storage Liquide Solution *Mycoplasma spp.* provided in the kit, which contains a suitable composition for preserve the conservation of fastidious microorganisms such as those detectable with this kit.
The samples of the respiratory tract should not be stored in conventional media for more than 24 hours [18-20] , and in these conditions are **NOT** recommended for use with this kit.

Sample processing: The sample must be handled according to the procedure established in each laboratory and must be prepared, stained and inoculated properly taking in account the type of sample received, its clinical diagnosis and the physician's prescription. Depending on the type of sample, the laboratory should evaluate and determine the need for centrifugation, homogenization or other procedure before its inoculation.

The Storage Liquide Solution *Mycoplasma spp.* allows the conservation of the sample at room temperature for 24 hours, 3 days at 4 ° C, or freezing at -20 / -70 ° C ([18 - 20]). Although the samples of the respiratory tract can sometimes be polymicrobial, the Storage Liquide Solution *Mycoplasma spp.* contains inhibitors and is selective for *Mycoplasma spp*., It is up to the microbiologist to use the advantages of the Storage Liquide Solution *Mycoplasma spp.* for further studies.

It is recommended to analyze the results obtained with MYCO PNEUMO ONE together with the traditional methods and complementary tests established in each laboratory. This kit combines the utility of the use of selective and / or chromogenic media with the possibility of carrying out any type of confirmation using the contents of the well where bacterial growth occurs and is observed.

Note: Other samples of the respiratory tract or related to an associated infectious process may be applied to the present identification system[17-20]. In the case of nasopharyngeal or pharyngeal exudate samples, the guidelines established for the determination of agents responsible for Respiratory Infections must be followed
The result obtained with this system should not be the sole diagnostic criterion, other clinical criteria or additional tests established by the clinical physician must be taken into account.

6.4 Reading Procedure and Interpretation
Carefully read the present Instructions for use, and carry out the complementary tests indicated.
If, after a 48-hour incubation, no positive reactions are observed in wells 1 to 16, the plate should be incubated and readings should be made every 24 hours, for a total of 5-7 days. All readings are made visually.

7. INTERPRETATION OF RESULTS
The results are made evident by the change in color that occurs in the wells, based on the reactions of the chemical or chromogenic components specifically formulated for each microorganism.
The interpretation is realized through the analysis of the reactions that take place in different wells.

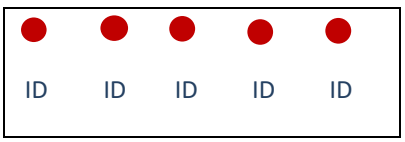
The wells included in the identification panel for the determination of antimicrobial susceptibility have been prepared according to the established lines and have been defined in relation to the antimicrobials suggested by the group of experts consulted in relation to the treatment of the microorganisms included in the the present kit ([9, 10, 18, 20]). However these can be modified in relation to previous requests or recommendations and in relation to the use of the same. The interpretation of the results is made by analyzing the reactions that occur in various wells (See the Table for the Interpretation of the Results)

Presumptive identification of *Mycoplasma pneumoniae/Mycoplasma spp.*

Presence of *Mycoplasma pneumoniae*

Presence of *Mycoplasma pneumoniae*

Presence of *M.pneumoniae/ Mycoplasma spp.*
(*Mycoplasma penetrans, Mycoplasma fermentans*)



Negative results for *Mycoplasma pneumoniae*

ANTIBIOTIC SUSCEPTIBILITY TEST

• A color change to yellow in the field of the antibiogram indicates the presence of bacterial growth and is therefore an index of resistance.
• In the case in which the color of one or more wells turns red, the growth of the test strain will be inhibited and therefore must be interpreted as sensitive.
• Antibiotic concentrations in the wells were prepared according to the guidelines established by CLSI [6] for *Mycoplasma pneumoniae* susceptibility testing
• Organisms susceptible to tetracycline are also susceptibile to doxycycline [6].

8. WARNINGS AND PRECAUTIONS

1. For professional *in vitro* diagnostic use only.
2.Samples should be treated as potentially infectious and tests should only be performed by qualified personnel.
3. Open only the tests necessary to perform the test.
4. Do not use expired devices.
5. Do not use components of any other type of kit.
6. Wear protective clothing and disposable gloves when handling reagents and clinical specimens. Wash your hands thoroughly after performing the test.
7. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
8. Dispose of all samples and materials used to perform the test as bio-hazardous waste.
9. Carefully read these Instructions for Use

9.LIMITATIONS

-Samples taken after starting the antimicrobial treatment. A single dose of antibiotic may invalidate the results of the test for detection of agents included in this kit [1] [4].
- Inadequate collection and storage of the sample (Paragraph 6.3).
- Inadequate sample (Paragraph 6.3).
-Personnel not employed or trained in microbiology.
- Read these Instructions for Use carefully before carrying out the test in order to avoid errors

10. QUALITY CONTROL

Each batch of MYCO PNEUMO ONE is subjected to a rigorous quality control with different strains. The reference strains are used both for the positive reactions of the different wells as to prove the good functioning of the media formulations of the different wells in case of any non-specific reactions [10] [11] [12].

To perform quality control, the following reference strains (including cross-reactions) are recommended:

Mycoplasma buccale ATCC 23636,
Mycoplasma hominis ATCC 23114,
Mycoplasma orale ATCC 23714
Mycoplasma pirum ATCC 25960
Mycoplasma pneumoniae ATCC 15531
Mycoplasma salivarium ATCC 23064
Mycoplasma fermentans ATCC 19989
Haemophilus influenzae ATCC 49247
Streptococcus pyogenes ATCC 19615
Streptococcus agalactiae ATCC 13813
Moraxella catarrhalis ATCC 25238
Staphylococcus aureus ATCC 25923
Enterobacter aerogenes ATCC 13048
Candida albicans ATCC 10231
Pseudomonas aeruginosa ATCC 27853
Klebsiella pneumoniae ATCC 13883
Enterococcus faecalis ATCC 29212

Each laboratory must establish an internal quality control.

11.STORAGE AND CONSERVATION

Store at 2-8 ° C in its original packaging. Do not store near heat sources and avoid extreme temperature variations. In 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.

12. BIBLIOGRAPHY

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COLORIMETRIC TABLE OF RESULTS

COLORIMETRIC REACTION

INTERPRETATION OF WELL 1

The well 1 allows the growth of *M. hominis* and *M. pneumoniae /M.spp.* The color of the well without inoculation is orange. A red color change in 24/48 hours means growth of *M. hominis* and, a change in color to yellow after 48 hours means growth of *M. pneumoniae / M.spp*

1	*M.pneumoniae/M.spp*	GIALLO/ARANCIO TRASPARENTE	ROSSO
		ROSSO	GIALLO/ARANCIO TRASPARENTE

INTERPRETATION OF WELLS FROM 2 TO 15

WELL	DESCRIPTION	POSITIVE	NEGATIVE
2	*M.pneumoniae/M.spp*	YELLOW/TRANSPARENT ORANGE	RED
3	*M.pneumoniae/M.spp*	YELLOW/TRANSPARENT GREEN	RED
4	*M.pneumoniae/M.spp*	YELLOW/TRANSPARENT GREEN	RED
5	*M.pneumoniae/M.spp*	YELLOW/TRANSPARENT GREEN	RED
6	Cross Reaction	TURBID RED/YELLOW	TRANSPARENT RED
7	Fungal Growth	TURBID YELLOW	SLIGHT YELLOW/TRANPARENT
8	Bact Growth	TURBIDITY	WHITE/SLIGHT YELLOW/ TRANSPARENT

AST

WELL	DESCRIPTION	RESISTANT	SUSCSEPTIBLE
9	LEV 1	YELLOW/TRANSPARENT ORANGE	RED
10	MXF 0,5	YELLOW/TRANSPARENT ORANGE	RED
11	TET 2	YELLOW/TRANSPARENT ORANGE	RED
12	E 0,5	YELLOW/TRANSPARENT ORANGE	RED
13	E 1	YELLOW/TRANSPARENT ORANGE	RED
14	AZ 0,5	YELLOW/TRANSPARENT ORANGE	RED
15	AZ 1	YELLOW/TRANSPARENT ORANGE	RED

INTERPRETATION OF WELL 16

The well 16 allows the growth of *M. hominis* and *M. pneumoniae /M.spp.* The color of the well without inoculation is orange. A red color change in 24/48 hours means growth of *M. hominis* and, a change in color to yellow after 48 hours means growth of *M. pneumoniae / M.spp*

16	*M. hominis*	RED	YELLOW/TRANSPARENT ORANGE

	M. pneumoniae/M.spp	YELLOW/TRANSPARENT ORANGE	RED

Simbols

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

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