A.R.I Well D-ONE®
System for the presumptive identification and antimicrobial/antifungal susceptibility test of the most common pathogenic agents in Acute Respiratory Infections

1.INTRODUCTION

Acute respiratory infections (ARI) are one of the major health problems in the world and one of the first causes of mortality and morbidity in pediatric patients. The microbiological diagnosis of acute respiratory infections seems to be difficult and different depending on the localization of the infectious process, its severity, the samples available and the patient's age. Additionally, the responsible etiological agents are various and epidemiology is different related with specific factors (community-acquired pneumonia, hospitalized patients, immunocompromised patients, geographic area, etc.)[(1)(2)(3)]. In developing countries, bacteria are the most isolated in severe pneumonia in untreated patients and etiological agents are a serious health problem in children and the elderly. Rapid diagnosis available today may involve the use of expensive tests, which although excellent methods, are not available in all hospital centers, or insufficient for involved agents.
It is common that in resource-poor regions is chosen to implement antimicrobial treatment as a priority, rather than spending resources in the microbiological diagnosis that requires an adequate infrastructure for its realization.
In the light of this, a system that allows rapidly growing, in addition to antimicrobial susceptibility test in just 18-24 hours, without additional equipment, can be a useful tool in the hands of the microbiologist and clinician.

2. PRINCIPLE

System composed of a polypropylene plate containing 32 conical wells for better viewing of the colorimetric reactions that occur as result of the growth of microorganisms in specially formulated media for selective culture of: *Streptococcus pneumoniae, Streptococcus pyogenes (Group A), Streptococcus agalactiae (Group B), Haemophilus influenzae, Haemophilus spp, Staphylococcus aureus, Mycoplasma spp., Mycoplasma pneumoniae, Pseudomonas spp., Candida spp., Candida albicans.*
A combined system of wells with specific formulations is also included to define the presumptive diagnosis of one or more microorganisms present in the sample. It is essential the general analysis of all the results obtained in the various wells by the microbiologist, to interpret the obtained results in the identification panel.
Microbiological diagnosis can be confirmed by serological tests, microscopic examination or culture of positive wells directly. The plate contains growth control wells and indicative wells that permit accelerated growth of other agents not herein described, for which serological diagnosis can be used, conventional or other available tests in the laboratory.
The antimicrobial susceptibility is designed for the determination of cut-off of sensitivity / resistance considering antimicrobials used most often for respiratory infections caused by agents most commonly isolated in these cases. Antimicrobial treatment of ARI follows protocol implemented in each care unit, the results obtained by the A.R.I. WELL D-ONE® ® system are approximate and should be consulted carefully based on the identified agent following these instructions and manual and table included in this instructions leaflet.

3. A.R.I. WELL D-ONE® KIT CONTENTS (REF. MS01293)

10 Identification panels	(REF. MS01293)
10 x 10 mL Physiological Saline Sterile solution	(REF. MS01304)
1 x 1 mL Reagent A (Pyr Reagent)	(REF. MS01307)

4. REAGENTS REQUIRED BUT NOT PROVIDED

Liquid Sterile Paraffin	(REF. MS01316)
Enriched Culture-ONE Medium	(REF. MS01317)

Latex Reagent for *Haemophilus influenzae* (a-f / b)
Latex Reagent for *Streptococcus groups A, B, C, D, F* and *G*
Latex Reagent for *Streptococcus pneumoniae*
Latex Reagent for *Staphylococcus aureus/MRSA*
Slides for agglutination
Reagents for catalase test
Reagents for coagulase test
Test for Oxidase determination
Latex reagents or Cards for identification of *Streptococcus* ß hemolytic.
Gram staining Reagent
General Laboratory Equipment

5. COMPOSITION OF TEST PLATE

Well 1: Selective Medium for the isolation of *Streptococcus spp./Staphylococcus spp.*
Well 2: Selective Medium for presumptive identification of *Streptococcus pneumoniae*
Well 3:Selective Medium for presumptive identification of *Streptococcus pneumoniae*
Well 4:Selective Medium for presumptive identification of *Streptococcus pyogenes*
Well 5:Selective Medium for presumptive identification of *Streptococcus pyogenes*
Well 6: Selective Medium for presumptive identification of *Streptococcus agalactiae*
Well 7: Selective Medium for presumptive identification of *Haemophilus influenzae*
Well 8: Selective Medium for presumptive identification of *Haemophilus influenzae*
Well 9: Selective Medium for presumptive identification of Gram Negative bacteria
Well 10: Selective Medium for presumptive identification of *Staphylococcus aureus*
Well 11: Selective Medium for presumptive identification of *Staphylococcus aureus*
Well 12: Selective Medium for presumptive identification of *Mycoplasma spp.*
Well 13: Selective Medium for presumptive identification of *Mycoplasma spp.*
Well 14: Selective Medium for presumptive identification of *Mycoplasma spp.*
Well 15: Selective Medium for presumptive identification of *Mycoplasma spp*.
Well 16: Selective Medium for presumptive identification of *Pseudomonas spp*.
Well 17: Culture medium containing PTZ 128/4 µg/mL
Well 18: Culture medium containing CF 32 µg/mL
Well 19: Culture medium containing CRO 64 µg/mL
Well 20: Culture medium containing VAN 2 µg/mL
Well 21: Culture medium containing CD 0,5 µg/mL
Well 22: Culture medium containing AZM 32 µg/mL
Well 23: Culture medium containing E 8 µg/mL
Well 24: Culture medium containing GEN 16 µg/mL
Well 25: Culture medium containing OFX 8 µg/mL
Well 26: Culture medium containing SXT 4/76 µg/mL
Well 27: Culture medium for the isolation of *Candida spp.*
Well 28: Culture medium for the isolation of *Candida spp.*
Well 29: Culture medium containing FLC 64 µg/mL
Well 30: Culture medium containing AMB 2 µg/mL
Well 31: Culture medium containing KTC 1 µg/mL
Well 32: Culture medium containing ITC 1 µg/mL

6. ASSAY METHOD

6.1 SAMPLE COLLECTION AND PREPARATION

Throat swab, nasopharyngeal, bronchial secretions, transtympanic aspiration (myringotomy), external ear exudate, pleural puncture, transthoracic needle aspiration, transtracheal puncture, bronchoalveolar lavage, lung puncture, abscess aspirates, blood cultures:

For the best performance of this system the sample must be taken according to the methodology implemented in each hospital and prior to antimicrobial treatment.
The sample should be processed immediately after collection (paragraph 6.3).
Do not refrigerate or freeze. Protect from light and sun.
The A.R.I. WELL D-ONE® system should be used by trained personnel of microbiology.
For any questions on the procedure or interpretation contact immediately the CPM product specialist in your area or directly to our central office.

Whenever possible it is recommended that respiratory tract samples are cultured in conventional media such as Columbia blood agar prepared with sheep blood 5%.

6.2 TEST PROCEDURE

Take with a swab or sterile Pasteur pipette a portion of the sample obtained according to the standards and procedures established in each laboratory for sample collection (see paragraph 6.3).

In the case of pharyngeal and nasopharyngeal exudates, pre-incubation of the sample is recommended in 3 mL of Trypticase Soy Broth for two hours. Take 200 µL of sample and resuspend in physiological saline vial provided in the kit. From this suspension inoculate 150 µL in A.R.I. WELL D-ONE® panel.

The rest of the respiratory samples to be analyzed with A.R.I. WELL D -ONE ® system must be processed as established in each laboratory according to what is mentioned in section 6.3. The microbiologist must identify that sample should be inoculated directly in physiological saline solution and should enriched in liquid culture media according to the established methodology. In case that sample must be enriched, Enriched Culture-ONE Medium is recommended, however the microbiologist can choose their own enrichment medium.

Samples requiring enrichment (Enriched Culture-ONE Medium or Trypticase Soy Broth):

After incubation of the Enriched Culture-ONE Medium or Trypticase Soy Broth Medium, resuspend carefully 200 µL of culture medium in the physiological saline vial provided in the kit.

Samples that do not require pre-enrichment:

For samples where the microbiologist use swabs and do not require the enrichment in culture medium:

Insert the swab into the vial of saline solution supplied in the kit and leave immersed for 5 minutes to ensure the sample diffusion from the swab to the suspension of the vial, then shake the swab and press it against the walls of the vial in order to obtain a homogeneous suspension.

Some samples that not require enrichment and are obtained with material different from the traditional swabs, can be dispensed directly into the vial of physiological saline and resuspend carefully to obtain a homogeneous suspension, before the inoculation of the panel.

Inoculation of Identification Panel:

Once obtained a homogenous suspension of the sample in the physiological saline solution, add 150 µL of the obtained suspension in all wells of the panel.

Add two drops of sterile paraffin in wells number: 2, 3, 7, 8, 12, 13, 14, 15 and from well 17 to well 26.

Incubation

Incubate at 36 ± 1 ^{o}C for 24 hours, in traditional laboratory incubator.

The incubation may be extended up to 48 hours or even up to 5 days, considering the time required for the growth of microorganisms, included in the identification panel, such as *Candida spp and Mycoplasma spp.*, according to the recommendations of this leaflet.

For incubations greater than 48 hours, should be observed color changes only in the wells 12, 13, 14 and 15. Other changes in the rest of the panel are not valid.

6.3 RECOMMENDATIONS FOR THE COLLECTION AND PROCESSING OF SAMPLES

(Each laboratory should establish its own method for proper collection of samples to test). [1][4][7][9]

Collection of samples. Aspects to consider: [1][4][5]

- Consider the risk / benefit of sample collection for the patient, especially in the case of invasive samples.
- The sample should be transported in suitable containers.
- The sample collection must be performed under conditions of maximum asepsis.
- It should be collected an adequate amount of sample.

In case it is necessary to transport or store the sample until processing, it is recommended to use the Enriched Culture-ONE medium, but the use of standard transport and storage medium such as Amies or Stuart does not interfere with the results of this kit. The sample should be processed within two hours of collection. If the sample will be processed after this period, it is recommended to use the Enriched Culture –ONE medium, which contains a suitable composition to preserve the conservation of fastidious microorganisms such as some of those that are included in this kit. Samples of the respiratory tract in the usual conservation medium should not be kept for more than 24 hours [6][7][8]. The immediate processing of the sample is recommended.

Sample processing: it is necessary to follow the procedures established in each laboratory, as concerns the sample preparation, the realization of Gram staining and the inoculation in the culture media. In this approach should consider the type of sample received at the laboratory, the clinical diagnosis of the patient and the request made by the clinician.
Depending on the sample the laboratory uses particular procedure and determines whether or not it is required pretreatment, enrichment, centrifugation, homogenization or other procedure before inoculation. [1][4][7][9]

Clinical information is essential for the inoculated culture media; however the laboratory uses primary culture media that allow the isolation of the most frequent causative agents of infectious processes. [7][9] However, sometimes the clinical syndrome may be caused by unusual organisms whose isolation requires the use of specific culture media and / or selective media. The suspicion of the involvement of any of these unusual microorganisms must be communicated to the laboratory. Likewise, the laboratory must communicate to clinicians the microorganisms routinely investigated and which must be specified in the request.

In case of samples that include enrichment in culture media and Enriched Culture-ONE Medium is used, it can be incubated at 36 ± 1 º C for 18 hours, for subsequent microscopic observations, serological tests, conventional culture or internal laboratory quality controls. The medium compositions also permit freezing to -20/-70 °C, either for storage of sample and the obtained cultures [8]. Although respiratory tract sample can sometimes be polymicrobial, is also common to find the presence of a pure culture of a microorganism, the microbiologist has to evaluate the use of Enriched Culture ONE Medium for further studies.

It is recommended to analyze the obtained results by A.R.I. WELL D-ONE® together with traditional methods of microscopic staining and / or other cell counting and analysis of the sample and examinations established in each laboratory. In relation to this, this kit combines the utility of using selective and/or chromogenic media with serological detection, in the appropriate case.

Note: Other samples of respiratory tract or related with an associated process can be applied to this identification system[8]. For samples of nasopharyngeal or pharyngeal exudates follow the rules established for the determination of the causative agents of Upper Respiratory Infections, healthy carriers of potentially pathogenic agents, transient bacterial colonization, etc. The result obtained by this system should not be the only diagnostic criteria but it should take in consideration other additional clinical tests or criteria set by the clinician.

6.4 Reading Procedure and Interpretation

Verify growth in well 1.

Add one drop of Reagent A in the well 4, wait 1 minute, if the reaction is positive a red color is developed. If the reaction is negative, well remains yellow.

Observe all the reactions of 1 to 11 wells and well 16.

Carefully read the *INSTRUCTIONS MANUAL AND INTERPRETATION OF RESULTS* and perform additional tests as indicated.
If not evident positive reactions are observed in wells 1-11; 16, the plate should be incubated for additional 24 hours.

Catalase test is performed by taking a drop of the content of the culture medium of the well 1 and adding one drop of hydrogen peroxide 3%.

Coagulase test can be performed by the test tube or slide, taking a drop of the content of the culture medium of the well 1.

The growth of some wells for the different species of *Mycoplasma spp.*, can take up to 5 days.

All readings are performed visually.

For *Streptococcus spp.*, it should be noted that in case of bacterial tonsillopharyngitis the most common causative agent is *Streptococcus pyogenes* (group A), however if streptococcal tonsillopharyngitis is suspected and the presumptive diagnosis by this kit gives negative result for antigenic reaction of group A, it may perform a test of latex for the rest of the group antigens for *Streptococcus spp.,* using the culture obtained in the well 1 and the latex agglutination kit (A-G) according manufacturer's instructions, because other Beta Hemolytic *Streptococcus* groups can cause tonsillopharyngitis. This method is not recommended if the wells are positive for *Staphylococcus aureus*.

Inoculation of respiratory tract samples in Columbia Blood Agar with sheep blood 5% permit to observe the different types of hemolysis and other interpretive aspects that help the microbiologist for the best interpretation of aspects reported in the *INSTRUCTIONS MANUAL AND INTERPRETATION OF RESULTS*. However, the kit can be used independently by validation indicated in this leaflet.

7. INTERPRETATION OF RESULTS

The results are evidenced by color changes that occur in wells according to reactions due to chemical or chromogenic components contained in specific formulations for each microorganism.
The wells included in this kit for the determination of antimicrobial susceptibility have been established according to established standards [10][11][12] and have been defined in relation to antimicrobial suggested by a group of experts consulted about the treatment of microorganisms included in this kit.
The interpretation of results is done by analyzing the reactions that occur in different wells (See *Instructions Manual and Interpretation of Results*).

8. WARNING AND PRECAUTIONS

1. For professional and *in vitro* diagnostic use only, not to be used by the general public.
2. The samples have to be treated as potentially infectious and the test must be carried out only by trained personnel.
3. Do not open the sealed pouch unless ready to perform the assay.
4. Do not use expired devices.
5. Do not use components from any other type of test kit as a substitute for the components in this kit.
6. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash 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 specimens and materials used to perform the test as bio-hazardous waste.
9. Read carefully the IFU and Manual and Interpretation of Results

9. LIMITATIONS

Samples collected after starting antimicrobial treatment. A single dose of antimicrobial may invalidate the results of

the test in detection of exigent microbial agents as some of those included in this kit[1][4].
Inadequate collection and storage of the sample (paragraph 6.3).
Inadequate sample (paragraph 6.3).
Staff not trained adequately in microbiology.

Read with attention this Instruction for use prior to realize the test in order to avoid errors and read carefully the *Instructions Manual and Interpretation of Results*.

10. QUALITY CONTROL

The control strains are used for both positive reactions from different wells to check the proper functioning of the media formulations of the different wells before any nonspecific reactions[10][11][12].
In order to realize the quality control, it is recommended to use the following reference strains:

Haemophilus influenzae ATCC 49247
Streptococcus pneumoniae ATCC 49619
Streptococcus pyogenes ATCC 19615
Streptococcus agalactiae ATCC 13813
Moraxella catarrhalis ATCC 25238
Staphylococcus aureus ATCC 25923
Mycoplasma hominis ATCC 23114
Mycoplasma pneumoniae ATCC 15531
Enterobacter aerogenes ATCC 13048
Candida albicans ATCC 10231
Pseudomonas aeruginosa ATCC 27853
Klebsiella pneumoniae ATCC 13883
Enterococcus faecalis ATCC 29212
Staphylococcus lugdunensis ATCC 49576
Each laboratory should establish its own internal quality controls.

11. STORAGE AND CONSERVATION

Store at 2-8 ° C in its original package. Do not store near heat sources and avoid extreme temperature variations. Under these conditions the product is valid until the expiration date shown on label of primary and secondary box. Do not use after this date. Discard if there are signs of deterioration.

12.BIBLIOGRAPHY

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Index of symbols

REF Reference code LOT LOT number Expiration date

Temperature limitation/Store between IVD For *in vitro* diagnostic use

Do not reuse Fragile, handle with care Consult Instructions for Use

Manufacturer Contains sufficient for <n> tests

CE

Conform to the Directive 98/79/EC on *in vitro* diagnostic medical device

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