OYRON Well D-ONE
System for the presumptive identification and antimicrobial susceptibility test of most common microorganisms in urinary tract infections

1. INTRODUCTION

Urinary tract infections (UTI) are one of the most frequent infectious diseases both in the community and in hospitals. Not only represent a clinical problem, but also have an economic impact on healthcare costs that represent. Microbiological diagnosis has been modified in recent years, and actually it is recommended to consider the recount of colony-forming units together with clinical aspects (cystitis, asymptomatic bacteriuria), patient gender presence of urethral catheter etc.. For pregnant women the detection of *Streptococcus agalactiae* is very important for a specific treatment, in order to prevent neonatal sepsis that may be caused by this agent; over 95 % of UTIs are caused by a single bacterial species, *Escherichia coli* which causes about 75-95 % of episodes of acute uncomplicated cystitis. *Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumoniae , Streptococcus agalactiae , Enterobacter spp. and Enterococcus spp.* are responsible for the vast majority of the other urinary tract infections (1) (2) (3).

This system is designed to detect microorganisms in urine samples obtained in different ways.
The concentration (CFU / mL) is sometimes difficult to perform due to various factors, sometimes unnecessary in cases specific such as pregnant women, elderly, disabled patients under special treatment, clinical follow up previous infections, another cases. Based on the results obtained with the kit Oyron Well D-ONE, complementary tests, signs and symptoms, and the criteria of the clinician, the results of this kit and the decision of antimicrobial treatment and complementary tests will are taken into account.

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:
Escherichia coli, Streptococcus agalactiae, Staphylococcus aureus, Enterobacter spp., Enterococcus spp., Proteus/Providencia group, Pseudomonas spp., KES group (Klebsiella, Enterobacter, Serratia), Candida spp., Candida albicans.
Microbiological diagnosis can be confirmed by serological tests, microscopic examination or culture of positive wells directly.

3. OYRON WELL D-ONE KIT CONTENTS	(REF. MS01285)
10 Identification panel	(REF. MS01285)
10 x 10 mL Saline Solution	(REF. MS01304)
Reagent A (Naphthylamine) 1 x 1 mL	(REF. MS01304)
Reagent B (Sulfanilic Acid) 1 x 1 xmL	(REF. MS01311)

(Bring the Reagent B to room temperature, before use)

4. REAGENTS REQUIRED BUT NOT PROVIDED

Laboratory equipment.

5. COMPOSITION OF TEST PLATE

Well 1: Culture Medium for detection of NO_2
Well 2: Culture Medium for Growth Control
Well 3: Culture Medium for Gram Negative bacteria presumptive detection
Well 4: Selective Medium for Gram Positive bacteria presumptive detection
Well 5:Selective Medium for the presumptive identification of *Escherichia coli*
Well 6: Selective Medium for the presumptive identification of *Escherichia coli*
Well 7: Selective Medium for presumptive identification of *Streptococcus agalactiae*
Well 8: Selective Medium for presumptive identification of *Streptococcus agalactiae*
Well 9: Selective Medium for presumptive identification of *Pseudomonas spp.*
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 *Enterobacter spp.*
Well 13: Selective Medium for presumptive identification of *Enterobacter spp.*
Well 14: Selective Medium for presumptive identification of *Proteus/Providencia*
Well 15: Selective Medium for presumptive identification of *Proteus/Providencia*
Well 16: Selective Medium for presumptive identification of *KES group (Klebsiella, Enterobacter, Serratia)*
Well 17: Selective Medium for presumptive identification of *Enterococcus spp.*
Well 18: Selective Medium for presumptive identification *Candida albicans*
Well 19: Selective Medium for presumptive identification *Candida spp.*
Well 20: Culture medium containing Fosfomycin 256 µg/mL
Well 21: Culture medium containing Amikacin 32 µg/mL
Well 22: Culture medium containing Gentamicin 8 µg/mL
Well 23: Culture medium containing Piperacillin/Tazobactam 128/4 µg/mL
Well 24: Culture medium containing Cefoperazone 64 µg/mL
Well 25: Culture medium containing Cefotaxime 64 µg/mL
Well 26: Culture medium containing Ceftazidime 32 µg/mL
Well 27: Culture medium containing Ampicillin/Sulbactam 32/16 µg/mL
Well 28: Culture medium containing Nalidixic Acid 32 µg/mL
Well 29: Culture medium containing Ciprofloxacin 4 µg/mL
Well 30: Culture medium containing Levofloxacin 8 µg/mL
Well 31: Culture medium containing Amoxicillin/Clavulanic Acid 32/16 µg/mL
Well 32: Culture medium containing Cotrimoxazol 8 µg/mL

6. ASSAY METHOD

6.1 SAMPLE COLLECTION AND PREPARATION

Samples:
Urine, suprapubic aspirations, urinary catheterization.
The sample must be obtained according to the procedure established in each hospital.
The urine sample must correspond with the first morning urine.
For children under 5 years follow the recommended procedure to avoid contamination of specimens. Urine samples obtained by catheterization or suprapubic aspiration are obtained according to medical prescriptions and trained personnel.
Specimens should be obtained before starting antimicrobial treatment or after at least three days to complete the same treatment.

For the best performance of this system the sample must be taken aseptically according to the methodology implemented in each hospital center. The sample should be processed immediately after collection; if that is not possible, specimens may be refrigerated at 2 to 8 °C for up to 24 hours and not more. Protect from light and sun.

The results obtained with OYRON Well D-ONE method should be compared to the traditional methods of analysis of the urine sample established in each laboratory.

6.2 TEST PROCEDURE
Take 200 µL (4 drops) of the sample and dilute in the saline solution vial provided in the kit.
Homogenize the solution with a Pasteur pipette or automatic pipette.
Aliquot 150 µL (3 drops) of the suspension obtained in each of the 32 wells of the kit.
Incubate at 37 °C for 24 hours (for *Candida spp.* 48 hours)
In the case of well 1 add 1 drop of Reagent A and 1 drop of Reagent B and wait for 5 minutes the color change.
In the case of well 19 a microscopic observation (40X) of the growth of chlamydospores and / or hyphae is recommended.

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 results obtained with OYRON WELL D-ONE should be analyzed together with the results obtained with traditional methods of microscopic staining and / or cell counting and other analysis of the sample, and examinations established in each laboratory.
The results must be qualified and evaluated according to the recommendations of this IFU.

For the interpretation of results, read carefully the *Instruction manual and interpretation of results* and the Reaction Table attached.

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. LIMITATIONS

Samples collected after antimicrobial treatment.
Samples collected inadequately.
Urine samples containing two or more microorganisms may cause different color change in identification wells and in antimicrobial susceptibility wells.
Read with attention this Instruction for use prior to realize the test in order to avoid errors.
The test have to be interpreted by microbiologist specialist.

10. QUALITY CONTROL

Each batch of OYRON Well D-ONE is submitted to a rigorous quality control with the following bacterial reference strains:
Escherichia coli ATCC 25922
Staphylococcus aureus ATCC 25923
Proteus mirabilis ATCC 25933
Enterococcus faecalis ATCC 19433
Candida albicans ATCC 10231
Candida krusei ATCC 6258
Candida glabrata ATCC 90030
Candida tropicalis ATCC 750
Enterococcus faecalis ATCC 29212
Enterobacter spp ATCC 13047 – ATCC 13048
Staphylococcus saprophyticus ATCC 15305
Pseudomonas aeruginosa ATCC 27853
Candida albicans ATCC 10231
Klebsiella pneumoniae ATCC 13883
Streptococcus agalatiae ATCC 13813

11. STORAGE

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 indicated on label. Do not use after this date. Discard if there are signs of deterioration.

12. BIBLIOGRAPHY

1. Edwards MS, Nizet V, Baker CJ. Group B Streptococcal Infections. In: Infectious Diseases of the Fetus and Newborn Infant, 7th ed, Remington JS, Klein JO, Wilson CB, et al (Eds), Elsevier Saunders, Philadelphia 2011:419.
2. Hooton TM, Bradley SF, Cardenas DD, Colgan R, Geerlings SE, Rice JC, et al. Diagnosis, prevention, and treatment of catheter-associated urinary tract infection in adults: 2009 International Clinical Practice Guidelines from the Infectious Diseases Society of America. Clin Infect Dis. 2010 Mar 1;50(5):625-63.
3.- Validazione del Sistema OYRON Well D-ONE in campioni da Infezioni delle vie Urinarie. Comparazione con metodi tradizionali. OYRON Well D-ONE Technical File, C.P.M. sas, 2013.
4. NCCLS. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. 2000. Fifth

Edition. Approved Standard M7-A5. Wayne, PA, USA.
5.NCCLS. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. 2002; 22(1). Approved Standard M2-A5, Wayne, PA, USA.

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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REACTIONS TABLE

WELL	COLORIMETRIC REACTION	
	POSITIVE	**NEGATIVE**
Well 1: Culture Medium for detection of NO_2	Red/Dark orange	Yellow
Well 2: Culture Medium for Growth Control	Turbid Yellow	
Well 3: Culture Medium for Gram Negative bacteria presumptive detection	Strong Yellow	White/Very slight yellow
Well 4: Selective Medium for Gram Positive bacteria presumptive detection	Turbid Red	White/very slight pink
Well 5: Selective Medium for the presumptive identification of *Escherichia coli*	Aqua Green	Slight Yellow
Well 6: Selective Medium for the presumptive identification of *Escherichia coli*	Blue	pink
Well 7: Selective Medium for presumptive identification of *Streptococcus agalactiae*	Green	Slight Yellow/Other colors
Well 8: Selective Medium for presumptive identification of *Streptococcus agalactiae*	Turbid Yellow	Transparent yellow
Well 9: Selective Medium for presumptive identification of *Pseudomonas spp.*	Turbid Aquamarine Green	White/other colors/Transparent
Well 10: Selective Medium for presumptive identification of *Staphylococcus aureus*	Violet/Dark purple	Slight Yellow/other colors
Well 11: Selective Medium for presumptive identification of *Staphylococcus aureus*	Black with precipitate	Slight Yellow/slight gray
Well 12: Selective Medium for presumptive identification of *Enterobacter spp.*	Turbid white	Slight yellow/transparent
Well 13: Selective Medium for presumptive identification of *Enterobacter spp.*	Turbid Pink	Slight Yellow/transparent/other colors
Well 14: Selective Medium for presumptive identification of *Proteus/Providencia*	Dark Brown/Black	Yellow
Well 15: Selective Medium for presumptive identification of *Proteus/Providencia*	Dark Brown/Black	Yellow
Well 16: Selective Medium for presumptive identification of *KES group (Klebsiella*, Enterobacter**, Serratia***)*	*green/**dark blue/ ***variable	Blue
Well 17: Selective Medium for presumptive identification of *Enterococcus spp.*	Black	Slight Yellow
Well 18: Selective Medium for presumptive identification *Candida albicans.*	Green	Slight Yellow
Well 19: Selective Medium for presumptive identification *Candida spp.*	Turbid Yellow	Slight Yellow

	ANTIBIOTIC SUSCEPTIBILITY TEST	
	RESISTENT	**SUSCEPTIBLE**
Well 20: Culture medium containing Fosfomycin 256 µg/mL	Turbid Beet Red	Clear white/ clear light pink
Well 21: Culture medium containing Amikacin 32 µg/mL	Turbid Beet Red	Clear white/ clear light pink
Well 22: Culture medium containing Gentamicin 8 µg/mL	Turbid Beet Red	Clear white/ clear light pink
Well 23: Culture medium containing Piperacillin/Tazobactam 128/4 µg/mL	Turbid Beet Red	Clear white/ clear light pink
Well 24: Culture medium containing Cefoperazone 64 µg/mL	Turbid Beet Red	Clear white/ clear light pink
Well 25: Culture medium containing Cefotaxime 64 µg/mL	Turbid Beet Red	Clear white/ clear light pink
Well 26: Culture medium containing Ceftazidime 32 µg/mL	Turbid Red	Clear white/ clear light pink
Well 27: Culture medium containing Ampicillin/Sulbactam 32/16 µg/mL	Turbid Beet Red	Clear white/ clear light pink
Well 28: Culture medium containing Nalidixic Acid 32 µg/mL	Turbid Red	Clear white/ clear light pink
Well 29: Culture medium containing Ciprofloxacin 4 µg/mL	Turbid Beet Red	Clear white/ clear light pink
Well 30: Culture medium containing Levofloxacin 8 µg/mL	Turbid Beet Red	Clear white/ clear light pink
Well 31: Culture medium containing Amoxicillin/Clavulanic Acid 32/16 µg/mL	Turbid Beet Red	Clear white/ clear light pink
Well 32: Culture medium containing Cotrimoxazol 8 µg/mL	Turbid Beet Red	Clear white/ clear light pink