FAECAL WELL D-ONE®

System for the presumptive identification and antibiotic susceptibility of pathogenic microorganisms of the intestinal tract.

1. INTRODUCTION

Intestinal and gastrointestinal infections are a fairly common problem that usually resolves within a few days, but in some cases, can lead to serious problems according to their pathogenicity, clinical condition and age of the patient.
Since the micro-organisms responsible for these clinical manifestations are numerous and complex it is important to identify the specific choice of the survey to be carried out, the method most suitable for diagnostic purposes and the appropriate therapy to be assigned depending on each specific case.
For this reason a system as FAECAL WELL D-ONE® allows both presumptive identification of gastrointestinal pathogens such as *Escherichia coli O157, Salmonella spp., Pseudomonsas spp., Shigella spp., Yersinia enterocolitica, Vibrio spp., Proteus / Providencia group., KES group, Enterobacter sakazakii, ESBL, KPC, Campylobacter spp*., found in fecal samples that susceptibility to antibiotics according to appropriate diagnostic criteria.

2. TEST PRINCIPLE AND CLINICAL SIGNIFICANCE

FAECAL WELL D-ONE® is a system composed of a polypropylene plate containing 32 conical wells for the better viewing of the colorimetric reactions that occur as a result of the growth of microorganisms, in faecal samples, responsible for gastrointestinal infections that often occur with a specific symptoms such as abdominal pain, diarrhea and in some cases, fever and vomiting.
The microbiological diagnosis can be confirmed by serological tests, microscopic examination or directly through cultivation of positive wells.

The plate is divided as follows:

The well No. 1 is specific for the growth control of enterobacteria, and then as a further confirmation of the presence of Gram-negative bacteria

Wells No. 2 and No. 3 allow the presumptive identification of *Escherichia coli O157*, known serotype entero-haemorrhagic *Escherichia coli O157* bacterium that contracts usually through the consumption of contaminated food. The infection can cause bloody diarrhea and, in severe cases, where children are exposed to particular risk, renal failure, anemia and dehydration, spontaneous bleeding, organ failure and mental changes in the elderly. Some of these patients develop permanent disabilities.

The wells No. 4-5-6-7 allow presumptive identification of *Salmonella spp*. The microorganism most commonly isolated after diarrheal events as a cause of food poisoning.
Infections caused by *Salmonella spp*. differ in forms with typhoid *Salmoenlla typhi* and *Salmonella paratyphi*, is generally responsible for typhoid fever that of enteric fever and forms non-typhoid, caused by *Salmonella typhimurium* and *Salmonella enteritidis*, responsible for clinical forms predominantly gastrointestinal manifestation.

The well No. 8 instead allows to identify presumptive *Pseudomonas spp.* frequent guest of the body (skin and digestive tract) but also often pathogen responsible for nosocomial infections, widely present in hospitals, where it is often due to real epidemics, especially among the elderly.

The wells No. 9 and 10 identify *Shigella spp.* is a species of Enterobacter frequently isolated as a cause of enteritis and enterocolitis.
Shigella sonnei with *Shigella flexneri* represent two of the most frequent species isolated as a cause of toxic food infection whose symptoms may be mild compared to *Shigella dysenteriae* certainly more virulent and dangerous and characterized by a more severe clinical picture that may result in severe dysentery (from hence its name of bacillary dysentery), nausea and sometimes vomiting, cramps and fever.
The virulence factor of this pathogen is given by its ability to produce the Shiga toxin (A and B) which acts as a cytotoxin capable of causing necrosis and ulceration.

The well No. 11 identifies *Yersinia enterocolitica*, an enterobacterium responsible of Yersiniosis that can cause a variety of symptoms depending on the age of the infected person. Infection with *Y. enterocolitica* occurs most often in children. The most common symptoms are fever, abdominal pain and diarrhea, often bloody. In children and adults, abdominal pain and fever may be the predominant symptoms. In a small percentage of cases, there may be complications such as skin rashes, joint pain, or the spread of bacteria in the bloodstream. Even *Yersinia enterocolitica* is among the pathogens causing toxic infection from food.

The wells No. 12 and No. 13, allow the identification of *Vibrio spp.* whose species are often pathogenic. Most of the strains that cause important diseases are associated with gastroenteritis. Pathogenic species normally known to be particularly virulent and dangerous include Vibrio cholerae (the causative agent of cholera), *V. parahaemolyticus* and *V. vulnificus. V. cholerae* is generally transmitted by contaminated water as well as from certain foods such as raw seafood. Outbreaks of *V. vulnificus* occur quite frequently in hot or humid areas. *V. parahaemolyticus* is manifested by symptoms similar to *V. cholerae* but can regress spontaneously within 2-3 days.

The wells No. 14 -15-16 allow to identify *Proteus / Providencia group.* Proteus is an enterobacterium that lives habitually in human gastrointestinal tract but may cause damage if it spreads to other locations such as the urinary tract where it can cause infections.

The wells No. 17-18 KES to identify the group KES (*Klebsiella, Enterobacter, Serratia*), microorganisms that can reside in the fecal flora but that can be both responsible for serious infections in other locations. For example *Klebsiella spp.* that despite being frequently retrieved in faeces are species that can cause respiratory infections often (*Klebsiella spp.),* and can also cause urinary infections and enteritis in immunocompromised individuals. *Enterobacter spp.,* lives normally in the intestinal flora but can cause urinary and extraintestinal infections. *Serratia spp.* regular guest of the human organism, in particular conditions, can become the cause of infections also generalized.

The well No. 19 identifies *Enterobacter sakazakii* opportunistic pathogen that deserves special attention as responsible for neonatal infections (especially meningitis and necrotizing enterocolitis) in many cases fatal.
Recent studies have shown its widespread mainly because isolated in food samples, in domestic environment, hospital and insects, and not least in powdered milk for infants, probably contaminated during production processes.

In this regard, the presence of even a modest charge of the microorganism in this type of food is enough to determine the risk of infection in children in the first months of life, especially if born under-weight or immunocompromised. Its detection and identification are therefore indispensable and fundamental for immediate diagnosis and preventive. The virulence factors related to the pathogenicity of *E. sakazakii* are not yet known. Some strains produce enterotoxin or similar substances to demonstrate a cytotoxic effect. However among the different strains studied, there are many differences, and some seem to lack the characteristics correlated with pathogenicity; and responsible for serious septicemia, neonatal meningitis and necrotizing enterocolitis in premature infants, in addition to support in rare cases forms of bacteremia and osteomyelitis in adults.

The well No. 20 allows the identification of *Enterobacteriaceae ESBL* (extended-spectrum beta-lactamase) or *Enterobacteriaceae* producing extended spectrum Beta Lactamase. They are then able to inactivate penicillins, cephalosporins narrow spectrum, many broad spectrum cephalosporins (cefotaxime and ceftazidime) and monobactamici (aztreonam).

The ESBL pathogens are:
Klebsiella pneumoniae, commensal organism of the intestinal tract and respiratory tract. May cause respiratory and urinary infections.
Escherichia coli, also commensal gut of humans and animals and can cause urinary tract infections, bacteremia and gastrointestinal infections.
Proteus mirabilis, commensal gut can lead to urinary tract infections, bacteremia and respiratory infections.
Enterobacter cloacae, commensal gut can lead to urinary tract infections, bacteremia, sepsis, respiratory infections and gastrointestinal infections.

The well No. 21 allows identification of KPC, or *Klebsiella pneumoniae carbapenemase*: Gram-negative bacteria are highly resistant to the drugs that cause infections associated with significant morbidity and mortality. A wide range of Gram negative bacteria, so not only K. pneumoniae, has important resistance mechanism to carbapenems.

The well No. 22 identifies *Campylobacter spp.* responsible for gastroenteritis of varying degrees, from mild diarrhea, fever and nausea, to more serious forms of colitis. Generally the infection resolves within a few days, and only rarely in the "at risk", such as children, the elderly and immunocompromised individuals, may occur post infectious complications such as arthritis and neurological disorders.

The wells from No. 23 to No. 30 allow the antimicrobial susceptibility test.

The wells No. 31 and No. 32 allows the presumptive identification of *Candida spp.,* a species of yeast that normally form part of the normal microbial flora of the skin, mouth, gastrointestinal tract and vagina. The best known is Candida albicans, which is responsible for many cases of vaginal disorders and more.

As a "guest" natural organism encounters situations favorable to the development of its pathogenicity in some specific cases that cause its incidence as clinical form.

That is to say that in its non-pathological form in non-immunocompromised, candidiasis is found generally only in the exposed parts and the moist body, such as:

- The oral cavity: the term is commonly used to indicate thrush stomatitis *Candida albicans*, a fungus that is charged to the oral mucosa.

- Armpits

- The vagina (vaginitis candida) as is necessary to remember that the Candida is a normal commensal of the vaginal flora.

In pathological form, *Candida spp.* creates a molecular structure very long, which penetrates through the intestinal mucosa blood flow.

The presence of candidiasis was found commonly in the digestive tract, especially in immunocompromised (AIDS patients or patients undergoing chemotherapy).

The test of susceptibility to antibiotics must be strictly interpreted in light of the results of the different wells of identification and vice versa.

3. FAECAL WELL D-ONE KIT CONTENTS (REF. MS01282)

10 Identification panels (REF. MS01282)
10 x 10 mL Saline Solution (REF. MS01304)

4.PANEL COMPOSITION

Well 1: Culture medium for the identification of Gram Negative Bacteria
Well 2: Culture medium for the presumptive identification of *Escherichia coli*
Well 3: Culture medium for the presumptive identification of *Escherichia coli O157*
Well 4: Culture medium for the presumptive identification of *Salmonella spp.*
Well 5: Culture medium for the presumptive identification of *Salmonella spp.*
Well 6: Culture medium for the presumptive identification of *Salmonella spp.*
Well 7: Culture medium for the presumptive identification of *Salmonella spp.*
Well 8: Culture medium for the identification of *Pseudomonas spp.*
Well 9: Culture medium for the presumptive identification of *Shigella spp.*
Well 10: Culture medium for the presumptive identification of *Shigella spp.*
Well 11: Culture medium for the identification of *Yersinia enterocolitica*
Well 12: Culture medium for the presumptive identification of *Vibrio spp.*
Well 13: Culture Selective medium for the identification of *Vibrio spp.*
Well 14: Culture medium for the presumptive identification of *Proteus/Providencia spp.*
Well 15: Culture medium for the presumptive identification of *Proteus/Providencia spp.*
Well 16: Culture medium for the presumptive identification of *Proteus/Providencia spp.*
Well 17: Culture medium for the presumptive identification of gruppo KES
Well 18: Culture medium for presumptive the identification of gruppo KES
Well 19: Culture medium for the identification of *Enterobacter sakazakii*
Well 20: Culture medium for presumptive the identification of ESBL
Well 21: Culture medium for the identification of KPC
Well 22: Culture medium for the presumptive identification of *Campylobacter spp.*

Well 23: Culture medium containing Colistin Sulfate 2 µg/mL
Well 24: Culture medium containing Gentamicin 16 µg/mL
Well 25: Culture medium containing Doxiciclin 16 µg/mL
Well 26: Culture medium containing Levofloxacin 8 µg/mL
Well 27: Culture medium containing Sulfametoxazole 8 µg/mL
Well 28: Culture medium containing Piperacillin/Tazobactam 128/4 µg/mL
Well 29: Culture medium containing Ciprofloxacin 4 µg/mL
Well 30: Culture medium containing Tobramicin 8 µg/mL
Well 31: Culture Selective medium for the identification of *Candida spp.*
Well 32: Culture Selective medium for the identification of *Candida spp.*

5. EXECUTION OF TEST

COLLECTION AND PREPARATION OF THE SAMPLE

Samples: Stool, rectal swab

The sample must be taken in accordance with the procedure established in each laboratory.

Feces:
-Take 1 g (solid stool) or 500 µL (liquid stool) sample and dilute it in a tube containing 5 mL of saline and resuspend carefully. Let stand 5 minutes.
-Homogenize the suspension (solution A) with a Pasteur pipette or an automatic pipette. Aliquot 150 µL of the obtained solution A in wells from 1 to 22. Take 200 µL of solution A and inoculate into the vial of physiological saline supplied in the kit (solution B). Inoculate 150 µL of solution B in the wells from 23 to 32.
-Add 2 drops of liquid paraffin in wells 6,9,22
-Incubate At 37 ° C for 24-48 hours.

Rectal swab
-Immerse the swab in saline solution vial contained in the kit.
-Press carefully the swab against the wall of the vial so that the material is adequately homogenized.
-Homogenize the solution with a Pasteur pipette or an automatic pipette. Aliquot 150 µL of the obtained suspension in wells from 1 to 32.
-Add 2 drops of liquid paraffin in wells 6,9,22.
-Incubate At 37 ° C for 24-48 hours.

6. INTERPRETATION OF RESULTS

The results are indicated by the colorimetric reactions that occur in wells as indicated in the attached table.

7. 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

8. LIMITATIONS

-Samples collected after starting antimicrobial treatment.
-Inadequate collection of the sample
-Staff not trained adequately in microbiology.

Read with attention this Instruction for use prior to realize the test in order to avoid errors

9. QUALITY CONTROL

Each batch of FAECAL WELL D-ONE® is submitted to a rigorous quality control with the following bacterial reference strains.

Escherichia coli ATCC 25922
Escherichia coli ATCC 43895
Proteus mirabilis ATCC 25933
Enterococcus faecalis ATCC 19433
Enterococcus faecalis ATCC 29212
Candida albicans ATCC 10231
Enterobacter cloacae ATCC 13047
Enterobacter aerogenes ATCC 13048
Enterobacter sakazakii ATCC 29544
Pseudomonas aeruginosa ATCC 27853
Klebsiella pneumoniae ATCC 13883
Salmonella typhimurium ATCC 14028
Salmonella enterica ATCC 39926
Shigella spp. ATCC 9905/ATCC 15391/ATCC 25931/ATCC 13313
Yersinia enterocolitica ATCC 9610
Campylobacter jejuni ATCC 29428
Vibrio parahaemolyticus ATCC 17802
Vibrio cholerae ATCC 39315

10. 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.

11. BIBLIOGRAPHY

1. DuPont, H.L. Approach to the patient with suspected enteric infection. Goldman L., Schafer AI, ed. Goldman's Cecil Medicine. 24th ed. Philadelphia, PA: Saunders Elsevier; 2011

2. Semrad C.E. Approach to the patient with diarrhea and malabsorption. In: Goldman L, Schafer AI, eds. Goldman's Cecil Medicine. 24th ed. Philadelphia, PA: Saunders Elsevier; 2011:chap 142.

3. Hines J., Nachamkin I. Effective use of the clinical microbiology laboratory for diagnosing diarrhoeal diseases. Clin Infect Dis 1996;23:1292–301

4. D. Drudy, N. R. Mullane, T. Quinn, P. G. Wall, S. Fanning. *Enterobacter sakazakii:* An Emerging Pathogen in Powdered Infant Formula. Clin Infect Dis. 2006 Apr 1;42(7):996-1002.

5. Validazione del Sistema FAECAL WELL D-ONE in campioni fecali. Comparazione con metodi tradizionali. FAECAL Well D-ONE Technical File, C.P.M. sas, 2013.

6.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

7.CLSI. Clinical and Laboratory Standards Institute. M100-S24—Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement, January 2014

WELLS	COLORIMETRIC REACTION	
	POSITIVE	**NEGATIVE**
Well 1: Culture medium for the identification of Gram Negative Bacteria	TURBID OR TRANSPARENT YELLOW	TURBID OR TRANSPARENT WHITE
Well 2: Culture medium for the identification of *Escherichia coli*	GREEN	TURBID OR TRANSPARENT WHITE
Well 3: Culture medium for the identification of *Escherichia coli O157*	MAUVE	TURBID WHITE/VARIABLE
Well 4: Culture medium for the identification of *Salmonella spp.*	BLACK	TURBID WHITE
Well 5: Culture medium for the identification of *Salmonella spp.*	DARK VIOLET	TURBID WHITE/OTHER COLOR
Well 6: Culture medium for the identification of *Salmonella spp.*	BLACK	YELLOW/OTHER COLOR
Well 7: Culture medium for the identification of *Salmonella spp.*	BLACK	YELLOW/OTHER COLOR
Well 8: Culture medium for the identification of *Pseudomonas spp.*	GREEN/TURBID GREEN	WHITE/TURBID WHITE
Well 9: Culture medium for the identification of *Shigella spp.*	YELLOW	BLACK/OTHER COLOR
Well 10: Culture medium for the identification of *Shigella spp.*	TURBID RED	BLACK/GREEN/VARIABILE
Well 11: Culture medium for the identification of *Yersinia enterocolitica*	MAUVE	TURBID WHITE/OTHER COLOR
Well 12: Culture medium for the identification of *Vibrio spp.*	AQUA GREEN	WHITE
Well 13: Culture Selective medium for the identification of *Vibrio spp.*	TURBID YELLOW/TURBID WHITE	GREEN
Well 14: Culture medium for the identification of *Proteus/Providencia spp.*	TURBID BLACK/DARK TURBID GREY	WHITE/TURBID WHITE
Well 15: Culture medium for the identification of *Proteus/Providencia spp.*	TURBID BLACK/DARK GREY	WHITE/TURBID WHITE
Well 16: Culture medium for the identification of *Proteus/Providencia spp.*	TURBID BLACK/DARK GREY	WHITE/TURBID WHITE
Well 17: Culture medium for the identification of gruppo KES	TURBID RED	TRANSPARENT WHITE/TURBID WHITE
Well 18: Culture medium for the identification of gruppo KES	TURBID GREEN	DARK GREY/TURBID-TRANSPARENT WHITE
Well 19: Culture medium for the identification of *Enterobacter sakazakii*	BLUE	MAUVE
Well 20: Culture medium for the identification of ESBL	TURBID BEET RED	WHITE/VIOLET/ PINK TRANSPARENT
Well 21: Culture medium for the identification of KPC	TURBID BEET RED	WHITE/VIOLET/ PINK TRANSPARENT
Well 22: Culture medium for the identification of *Campylobacter spp.*	TURBID BEET RED	WHITE OR PINK TRANSPARENT
Well 23: Culture medium containing Colistin Sulfate 2 µg/mL	TURBID BEET RED	WHITE / SLIGHT PINK TRANSPARENT
Well 24: Culture medium containing Gentamicin 16 µg/mL	TURBID BEET RED	WHITE / SLIGHT PINK TRANSPARENT
Well 25: Culture medium containing Doxiciclin 16 µg/mL	TURBID BEET RED	WHITE / SLIGHT PINK TRANSPARENT
Well 26: Culture medium containing Levofloxacin 8 µg/mL	TURBID BEET RED	WHITE / SLIGHT PINK TRANSPARENT
Well 27: Culture medium containing Sulfametoxazole 8 µg/mL	TURBID BEET RED	WHITE / SLIGHT PINK TRANSPARENT
Well 28: Culture medium containing Piperacillin/Tazobactam 128/4 µg/mL	TURBID BEET RED	WHITE / SLIGHT PINK TRANSPARENT
Well 29: Culture medium containing Ciprofloxacin 4 µg/mL	TURBID BEET RED	WHITE / SLIGHT PINKTRANSPARENT
Well 30: Culture medium containing Tobramicin 8 µg/mL	TURBID BEET RED	WHITE / SLIGHT PINK TRANSPARENT
Well 31: Culture Selective medium for the identification of *Candida spp.*	GREEN	WHITE/TURBID WHITE
Well 32: Culture Selective medium for the identification of *Candida spp.*	YELLOW TURBID	TRANSPARENT

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

C.P.M. sas – Via degli Olmetti, 5 – 00060 (Zona Ind.le) Formello, (ROMA), Italia
Tel. +39/06.90.400.126 +39/06.90.75.531 - Fax. +39/06.90.405.270 –
www.cpmsas.it - cpm@cpmsas.it