

# DISKS FOR ANTIBIOTIC SUSCEPTIBILITY TESTING

## 50 DISKS CARTRIDGE

STUDY OF SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS.



### 1- CLINICAL VALUE

These disks are used to semi quantitatively evaluate the in vitro susceptibility to antimicrobial agents of rapidly growing bacteria and several difficult species by an agar diffusion method.

This method is based on a standardized procedure published by the WHO<sup>(1)</sup> and adopted as consensual standard by the CLSI<sup>(2,3)</sup> CA-SFM<sup>(4)</sup> and EUCAST<sup>(5)</sup> (it is periodically revised).

**Consult the most recent CLSI<sup>(2,3)</sup>, CA-SFM<sup>(4)</sup>, EUCAST<sup>(5)</sup> documents for guidelines concerning antibiotic susceptibility testing and interpretation of the results.**

### 2- PRINCIPLE

Paper disks impregnated with a defined concentration of antimicrobial agent are deposited on the surface of an appropriate medium<sup>(A)</sup> previously inoculated with a calibrated inoculum<sup>(A)</sup> of pure and fresh culture of the bacterial strain to be tested. After incubation<sup>(A)</sup>, the Petri dishes are examined and the zones of inhibition around the disks are measured and compared to critical values<sup>(A)</sup> for the various antimicrobial agents tested, in order to determine the clinical category of susceptibility (resistant, intermediate, susceptible...). The diameter of the zone of inhibition is proportional to the susceptibility of the bacterial strain tested

<sup>(A)</sup>According to current guidelines CLSI<sup>(2,3)</sup> CA-SFM<sup>(4)</sup> EUCAST<sup>(5)</sup>.

### 3- PRESENTATION

Bio-Rad disks are 6.5 mm disks made from superior quality absorbent paper and impregnated with precise concentrations of antimicrobial agents. The disks are clearly identified by a code, comprising 1 to 3 letters, printed on each side of the disk (see table 1). Bio-Rad disks are supplied in cartridges of 50 disks packaged in watertight containers containing a desiccant.

**Table 1 : Bio-Rad disks for antibiotic susceptibility testing**

|                               | DISK CONTENT   | SYMBOL  | PACK SIZE    | ORDER N° |
|-------------------------------|----------------|---------|--------------|----------|
| Amikacin                      | 30 µg          | AN      | 4 x 50 Disks | 66148    |
| Amoxicillin                   | 25 µg          | AMX     | 4 x 50 Disks | 66138    |
| Amoxicillin + Clavulanic Acid | 20 + 10 µg     | AMC     | 4 x 50 Disks | 66178    |
| Ampicillin                    | 2 µg           | AMN 2   | 4 x 50 Disks | 67288    |
|                               | 10 µg          | AM      | 4 x 50 Disks | 66128    |
| Ampicillin + Sulbactam        | 10 + 10 µg     | SAM     | 4 x 50 Disks | 67018    |
| Azithromycin                  | 15 µg          | AZM     | 4 x 50 Disks | 67008    |
| Aztreonam                     | 30 µg          | ATM     | 4 x 50 Disks | 66928    |
| Bacitracin                    | 130 µg / 10 UI | B       | 4 x 50 Disks | 66158    |
| Carbenicillin                 | 100 µg         | CB 100  | 4 x 50 Disks | 66198    |
| Cefaclor                      | 30 µg          | CEC 30  | 4 x 50 Disks | 67498    |
| Cefalexin                     | 30 µg          | CN      | 4 x 50 Disks | 66208    |
| Cefamandole                   | 30 µg          | MA      | 4 x 50 Disks | 66238    |
| Cefazolin                     | 30 µg          | CZ      | 4 x 50 Disks | 66258    |
| Cefepime                      | 30 µg          | FEP     | 4 x 50 Disks | 66098    |
| Cefixime                      | 5 µg           | CFM 5   | 4 x 50 Disks | 67588    |
|                               | 10 µg          | CFM 10  | 4 x 50 Disks | 66418    |
| Cefoperazone                  | 75 µg          | CFP 75  | 4 x 50 Disks | 67618    |
|                               | 30 µg          | CFP 30  | 4 x 50 Disks | 66298    |
| Cefoperazone + Sulbactam      | 75 + 30 µg     | SCF 105 | 4 x 50 Disks | 66734    |
| Cefotaxime                    | 5 µg           | CTX 5   | 4 x 50 Disks | 67718    |
|                               | 30 µg          | CTX     | 4 x 50 Disks | 66368    |
| Cefotetan                     | 30 µg          | CTT     | 4 x 50 Disks | 66428    |
| Cefoxitin                     | 30 µg          | FOX     | 4 x 50 Disks | 66228    |
| Cefpirome                     | 30 µg          | CPO     | 4 x 50 Disks | 66468    |
| Cefpodoxime                   | 10 µg          | CPD     | 4 x 50 Disks | 66918    |
| Cefprozil                     | 30 µg          | CPR     | 4 x 50 Disks | 66488    |
| Cefsulodin                    | 30 µg          | CFS     | 4 x 50 Disks | 66938    |
| Ceftazidime                   | 10 µg          | CTZ 10  | 4 x 50 Disks | 67298    |
|                               | 30 µg          | CAZ     | 4 x 50 Disks | 66308    |
| Ceftibuten                    | 30 µg          | CTB 30  | 4 x 50 Disks | 67638    |

|                           | DISK CONTENT   | SYMBOL  | PACK SIZE    | ORDER N° |
|---------------------------|----------------|---------|--------------|----------|
| Ceftriaxone               | 30 µg          | CRO     | 4 x 50 Disks | 66188    |
| Cefuroxime                | 30 µg          | CXM     | 4 x 50 Disks | 66358    |
| Cephalotin                | 30 µg          | CF      | 4 x 50 Disks | 66218    |
| Chloramphenicol           | 30 µg          | C       | 4 x 50 Disks | 66278    |
| Ciprofloxacin             | 5 µg           | CIP     | 4 x 50 Disks | 68648    |
| Clarithromycin            | 15 µg          | CLR     | 4 x 50 Disks | 67058    |
| Clindamycin               | 2 µg           | CM      | 4 x 50 Disks | 66328    |
| Colistin                  | 10 µg          | CS 10   | 4 x 50 Disks | 67268    |
|                           | 50 µg          | CS 50   | 4 x 50 Disks | 66348    |
| Doripenem                 | 10 µg          | DORI 10 | 4 x 50 Disks | 67348    |
| Doxycycline               | 30 µg          | DO      | 4 x 50 Disks | 66388    |
| Ertapenem                 | 10 µg          | ETP 10  | 4 x 50 Disks | 67518    |
| Erythromycine             | 15 µg          | E       | 4 x 50 Disks | 66448    |
| Flumequine                | 30 µg          | UB      | 4 x 50 Disks | 68918    |
| Fosfomycin                | 50 µg          | FOS 50  | 4 x 50 Disks | 66458    |
|                           | 200 µg         | FOS 200 | 4 x 50 Disks | 67658    |
| Fusidic Acid              | 10 µg          | FA      | 4 x 50 Disks | 66518    |
| Gentamicin                | 10 µg          | GM      | 4 x 50 Disks | 66608    |
|                           | 15 µg / 10 UI  | GM      | 4 x 50 Disks | 66548    |
|                           | 30 µg          | GME 30  | 4 x 50 Disks | 67318    |
| Gentamicin (high load)    | 120 µg         | GEN 120 | 4 x 50 Disks | 67598    |
|                           | 500 µg         | GEN 500 | 4 x 50 Disks | 66578    |
| Imipenem                  | 10 µg          | IPM     | 4 x 50 Disks | 66568    |
| Isepamicin                | 30 µg          | ISP     | 4 x 50 Disks | 66838    |
| Kanamycin                 | 30 µg          | K       | 4 x 50 Disks | 66618    |
| Kanamycin (high load)     | 1mg            | KAN     | 4 x 50 Disks | 66628    |
| Levofloxacin              | 5 µg           | LVX     | 4 x 50 Disks | 66858    |
| Lincomycin                | 15 µg          | L       | 4 x 50 Disks | 66678    |
| Linezolid                 | 10 µg          | LZD 10  | 4 x 50 Disks | 67878    |
|                           | 30 µg          | LZD 30  | 4 x 50 Disks | 67388    |
| Mecillinam                | 10 µg          | MEC     | 4 x 50 Disks | 66768    |
| Meropenem                 | 10 µg          | MEM     | 4 x 50 Disks | 67048    |
| Metronidazole             | 4 µg           | MTR     | 4 x 50 Disks | 68908    |
| Mezlocillin               | 75 µg          | MZ      | 4 x 50 Disks | 66708    |
| Minocycline               | 30 µg          | MNO     | 4 x 50 Disks | 66728    |
| Moxalactam                | 30 µg          | MOX     | 4 x 50 Disks | 66698    |
| Moxifloxacin              | 5 µg           | MXF 5   | 4 x 50 Disks | 67098    |
| Mupirocin                 | 5 µg           | MUP     | 4 x 50 Disks | 67088    |
| Nalidixic Acid            | 30 µg          | NA      | 4 x 50 Disks | 68618    |
| Neomycin                  | 30 UI          | N       | 4 x 50 Disks | 66748    |
| Netilmicin                | 10 µg          | NET 10  | 4 x 50 Disks | 67798    |
|                           | 30 µg          | NET     | 4 x 50 Disks | 66758    |
| Nitrofurantoin            | 100 µg         | NIF 100 | 4 x 50 Disks | 67328    |
|                           | 300 µg         | FT      | 4 x 50 Disks | 68678    |
| Nitroxolin                | 20 µg          | NI      | 4 x 50 Disks | 68778    |
| Norfloxacin               | 10 µg          | NOR 10  | 4 x 50 Disks | 66338    |
|                           | 5 µg           | NOR 5   | 4 x 50 Disks | 68238    |
| Ofloxacin                 | 5 µg           | OFX     | 4 x 50 Disks | 68938    |
| Oxacillin                 | 1 µg           | OX1     | 4 x 50 Disks | 66888    |
|                           | 5 µg           | OX      | 4 x 50 Disks | 66848    |
| Oxolinic Acid             | 10 µg          | OA      | 4 x 50 Disks | 68628    |
| Pefloxacin                | 5 µg           | PEF     | 4 x 50 Disks | 68228    |
| Penicillin                | 1 IU           | P 1     | 4 x 50 Disks | 67788    |
|                           | 6 µg / 10 IU   | P       | 4 x 50 Disks | 67218    |
| Pipemidic Acid            | 20 µg          | PI      | 4 x 50 Disks | 68638    |
| Piperacillin              | 30 µg          | PIL 30  | 4 x 50 Disks | 68478    |
|                           | 75 µg          | PIP 75  | 4 x 50 Disks | 67258    |
|                           | 100 µg         | PIP 100 | 4 x 50 Disks | 67228    |
| Piperacillin + Tazobactam | 100 + 10 µg    | TZP 110 | 4 x 50 Disks | 67238    |
|                           | 75 + 10 µg     | TZP 85  | 4 x 50 Disks | 66498    |
|                           | 30 + 6 µg      | PTZ 36  | 4 x 50 Disks | 67338    |
| Polymixin                 | 50 µg / 300 UI | PB      | 4 x 50 Disks | 67248    |
| Pristinamycin             | 15 µg          | PT      | 4 x 50 Disks | 67278    |
| Quinupristin-Dalfopristin | 15 µg          | QD 15   | 4 x 50 Disks | 67528    |
| Rifampicin                | 5 µg           | RA 5    | 4 x 50 Disks | 66648    |
|                           | 30 µg          | RA 30   | 4 x 50 Disks | 67308    |


|                                 | <b>DISK CONTENT</b>    | <b>SYMBOL</b> | <b>PACK SIZE</b> | <b>ORDER N°</b> |
|---------------------------------|------------------------|---------------|------------------|-----------------|
| Sparfloxacin                    | <b>5 µg</b>            | SPX           | 4 x 50 Disks     | 66538           |
| Spectinomycin                   | <b>100 µg</b>          | SPT           | 4 x 50 Disks     | 68798           |
| Spiramycin                      | 100 µg                 | SP            | 4 x 50 Disks     | 67378           |
| Streptomycin                    | <b>10 µg</b>           | S             | 4 x 50 Disks     | 67418           |
| Streptomycin (high load)        | <b>300 µg</b>          | STR 300       | 4 x 50 Disks     | 67608           |
|                                 | 500 µg                 | STR 500       | 4 x 50 Disks     | 67428           |
| Sulfonamides                    | <b>300 µg</b>          | SSS 300       | 4 x 50 Disks     | 67578           |
|                                 | 200 µg                 | SSS 200       | 4 x 50 Disks     | 68408           |
| Teicoplanin                     | <b>30 µg</b>           | TEC           | 4 x 50 Disks     | 68948           |
| Telithromycin                   | <b>15 µg</b>           | TEL 15        | 4 x 50 Disks     | 67538           |
| Tetracycline                    | <b>30 µg</b>           | TE            | 4 x 50 Disks     | 67448           |
| Ticarcillin                     | <b>75 µg</b>           | TIC           | 4 x 50 Disks     | 67458           |
| Ticarcillin + Clavulanic Acid   | <b>75 + 10 µg</b>      | TCC           | 4 x 50 Disks     | 67468           |
| Tigecycline                     | <b>15 µg</b>           | TGC 15        | 4 x 50 Disks     | 67398           |
| Tobramycin                      | <b>10 µg</b>           | TM            | 4 x 50 Disks     | 67488           |
|                                 | 30 µg                  | TOB 30        | 4 x 50 Disks     | 67358           |
| Trimethoprim + Sulfamethoxazole | <b>1.25 + 23.75 µg</b> | SXT           | 4 x 50 Disks     | 68898           |
| Trimethoprim                    | <b>5 µg</b>            | TMP           | 4 x 50 Disks     | 68888           |
| Vancomycin                      | 5 µg                   | VA 5          | 4 x 50 Disks     | 67828           |
|                                 | <b>30 µg</b>           | VA            | 4 x 50 Disks     | 68928           |

\*CLSI disk content are indicated in bold type.

#### 4- STORAGE

The expiry date applies exclusively to disks contained in intact cartridges stored according to the manufacturer's instructions. The expiry date and batch number are indicated on each packaging (cartridge and container).

- Cartridges of disks must be stored in their containers at a temperature between +2°C and +8°C in a dry place.
- Container must be allowed to adjust to room temperature (18-30°C) before opening. After applying the disks, return unused cartridges to a temperature between +2°C and +8°C.
- Do not use disks after the expiry date. Do not use any cartridge of disks left at room temperature (18-30°C) for more than 8 hours without verifying an acceptable level of performance before continuing to use this cartridge <sup>(6)</sup>.
- If the cartridge remains in the distributor after dispatch, it is necessary to preserve at +2-8°C in a dry place **with desiccants inside**.

The stability of the disks, of open cartridges placed in distributors (preserved according to the recommendations with desiccants) was validated in routine conditions during 6 weeks, except for the antibiotic discs marked with the symbol , for which the stability in weeks is shown inside this symbol.

#### 5- MATERIAL REQUIRED BUT NOT SUPPLIED

- disk dispenser: 6-7 disks Ref. # 50294  
12-16 disks Ref. # 50295
- culture media according to current guidelines (CLSI<sup>(2,3)</sup>, CA-SFM<sup>(4)</sup>, EUCAST<sup>(5)</sup>)
- reagents
- bacterial strains for quality control • opacity control equivalent to the Mac Farland 0.5 standard
- laboratory equipment necessary for antibiotic susceptibility testing by the agar diffusion method.

#### 6- PRECAUTIONS

Follow the instructions of the current guidelines (CLSI<sup>(2,3)</sup>, CA-SFM<sup>(4)</sup>, EUCAST<sup>(5)</sup>). Always observe the current techniques and precautions concerning protection against microbiological hazards. After use, sterilize the cultures and all contaminated material.

#### 7- PROCEDURE

- Samples: disks must not be used for tests performed directly on biological samples. Refer to the current guidelines (CLSI<sup>(2,3)</sup>, CA-SFM<sup>(4)</sup>, EUCAST<sup>(5)</sup>) defining preparation of the inoculum from a pure, fresh culture.
- Refer to the instructions recommended by the CLSI<sup>(2,3)</sup>, the CA-SFM<sup>(4)</sup> or the EUCAST<sup>(5)</sup> for all steps of antibiotic susceptibility testing and interpretation of the results: the CLSI<sup>(2,3)</sup>, CA-SFM<sup>(4)</sup>, or EUCAST<sup>(5)</sup> propose standardised techniques for preparation of the inoculum, inoculation of Petri dishes, the choice and arrangement of test disks, the incubation temperature and incubation time. Good laboratory practice should also be applied at all times.

## 8- INTERPRETATION OF THE RESULTS

- Precisely measure the diameters of the zones of inhibition observed and refer to the critical diameters indicated by current guidelines (CLSI<sup>(2,3)</sup>, CA-SFM<sup>(4)</sup>, EUCAST<sup>(5)</sup>).
- A clinical category (intermediate resistant, susceptible or not susceptible...) is given to each micro-organism as a function of the observed diameter and the critical diameters for the antibiotic tested.
- These criteria of clinical categorization according to critical diameters are periodically revised by the CLSI<sup>(2,3)</sup>, CA-SFM<sup>(4)</sup>, EUCAST<sup>(5)</sup>.

## 9- PERFORMANCE/QUALITY CONTROL

The performances of antibiotic disks are systematically controlled by using the following strains:

- *Escherichia coli* ATCC 25922
- *Staphylococcus aureus* ATCC 25923
- *Pseudomonas aeruginosa* ATCC 27853
- *Streptococcus pneumoniae* ATCC 49619
- *Neisseria gonorrhoeae* ATCC 49226
- *Haemophilus influenzae* ATCC 49247
- *Enterococcus faecalis* ATCC 33186
- *Clostridium perfringens* ATCC 13124
- *Enterococcus faecalis* ATCC 29212
- *Clostridium sporogenes* ATCC 19404

The table 2 indicates the accepted limits for the diameters of inhibition obtained by the agar diffusion method for the reference strains indicated below.

|                               | Disk Content | Standard | Acceptable Zone Diameter (mm) Quality Control Limits |                                |                                    |                                    |                                    |
|-------------------------------|--------------|----------|--|--------------------------------|------------------------------------|------------------------------------|------------------------------------|
|                               |              |          | <i>E. coli</i><br>ATCC 25922                         | <i>S. aureus</i><br>ATCC 25923 | <i>P. aeruginosa</i><br>ATCC 27853 | <i>S. pneumoniae</i><br>ATCC 49619 | <i>H. influenzae</i><br>ATCC 49247 |
| Amikacin                      | 30 µg        | CLSI     | 19-26  | 20-26                          | 18-26                              |                                    |                                    |
| Amoxicillin                   | 25 µg        | Internal | 19-25  |                                |                                    |                                    |                                    |
| Amoxicillin + Clavulanic Acid | 20/10 µg     | CLSI     | 18-24  | 28-36                          |                                    |                                    |                                    |
| Ampicillin                    | 10 µg        | CLSI     | 16-22  | 27-35                          |                                    |                                    |                                    |
| Ampicillin + Sulbactam        | 10/10 µg     | CLSI     | 19-24  | 29-37                          |                                    |                                    |                                    |
| Azithromycin                  | 15 µg        | CLSI     |  | 21-26                          |                                    |                                    |                                    |
| Aztreonam                     | 30 µg        | CLSI     | 28-36  |                                | 23-29                              |                                    |                                    |
| Bacitracin                    | 10 UI        | Internal |  | 17-22                          |                                    |                                    |                                    |
| Carbenicillin                 | 100 µg       | CLSI     | 23-29  |                                | 18-24                              |                                    |                                    |
| Cefaclor                      | 30 µg        | CLSI     | 23-27  | 27-31                          |                                    |                                    |                                    |
| Cefalexin                     | 30 µg        | Internal | 18-23  | 25-30                          |                                    |                                    |                                    |
| Cefamandole                   | 30 µg        | CLSI     | 26-32  | 26-34                          |                                    |                                    |                                    |
| Cefazolin                     | 30 µg        | CLSI     | 21-27  | 29-35                          |                                    |                                    |                                    |
| Cefepime                      | 30 µg        | CLSI     | 31-37  | 23-29                          | 24-30                              |                                    |                                    |
| Cefixime                      | 10 µg        | Internal | 26-30  |                                |                                    |                                    |                                    |
| Cefixime                      | 5 µg         | CLSI     | 23-27  |                                |                                    |                                    |                                    |
| Cefoperazone                  | 30 µg        | Internal | 26-30  | 23-28                          | 21-25                              |                                    |                                    |
| Cefoperazone                  | 75 µg        | CLSI     | 28-34  | 24-33                          | 23-29                              |                                    |                                    |
| Cefoperazone + Sulbactam      | 75/30 µg     | Internal | 27-33  | 23-30                          |                                    |                                    |                                    |
| Cefotaxime                    | 30 µg        | CLSI     | 29-35  | 25-31                          | 18-22                              |                                    |                                    |
| Cefotetan                     | 30 µg        | CLSI     | 28-34  | 17-23                          |                                    |                                    |                                    |
| Cefoxitin                     | 30 µg        | CLSI     | 23-29  | 23-29                          |                                    |                                    |                                    |
| Cefpirome                     | 30 µg        | Internal | 29-35  | 26-32                          | 23-29                              |                                    |                                    |
| Cefpodoxime                   | 10 µg        | CLSI     | 23-28  | 19-25                          |                                    |                                    |                                    |
| Cefprozil                     | 30 µg        | CLSI     | 21-27  | 27-33                          |                                    |                                    |                                    |
| Cefsulodin                    | 30 µg        | Internal |  | 20-28                          | 25-32                              |                                    |                                    |
| Ceftazidime                   | 30 µg        | CLSI     | 25-32  | 16-20                          | 22-29                              |                                    |                                    |
| Ceftibuten                    | 30 µg        | CLSI     | 27-35  |                                |                                    |                                    |                                    |
| Ceftriaxone                   | 30 µg        | CLSI     | 29-35  | 22-28                          | 17-23                              |                                    |                                    |
| Cefuroxime                    | 30 µg        | CLSI     | 20-26  | 27-35                          |                                    |                                    |                                    |
| Chloramphenicol               | 30 µg        | CLSI     | 21-27  | 19-26                          |                                    |                                    |                                    |
| Ciprofloxacin                 | 5 µg         | CLSI     | 30-40  | 22-30                          | 25-33                              |                                    |                                    |
| Clarithromycin                | 15 µg        | CLSI     |  | 26-32                          |                                    |                                    |                                    |
| Clindamycin                   | 2 µg         | CLSI     |  | 24-30                          |                                    |                                    |                                    |
| Colistin                      | 10 µg        | CLSI     | 11-17  |                                | 11-17                              |                                    |                                    |
| Colistin                      | 50 µg        | CA-SFM   |  |                                | 17-22                              |                                    |                                    |

|                           | Disk Content | Standard | Acceptable Zone Diameter (mm) Quality Control Limits |                                |                                    |                                    |                                    |
|---------------------------|--------------|----------|--|--------------------------------|------------------------------------|------------------------------------|------------------------------------|
|                           |              |          | <i>E. coli</i><br>ATCC 25922                         | <i>S. aureus</i><br>ATCC 25923 | <i>P. aeruginosa</i><br>ATCC 27853 | <i>S. pneumoniae</i><br>ATCC 49619 | <i>H. influenzae</i><br>ATCC 49247 |
| Doxycycline               | 30 µg        | CLSI     | 18-24  | 23-29                          |                                    |                                    |                                    |
| Ertapenem                 | 10 µg        | CLSI     | 29-36  |                                | 13-21                              |                                    |                                    |
| Erythromycine             | 15 µg        | CLSI     |  | 22-30                          |                                    |                                    |                                    |
| Flumequine                | 30 µg        | Internal | 25-31  |                                |                                    |                                    |                                    |
| Fosfomycin                | 50 µg        | CA-SFM   |  | 24-35                          |                                    |                                    |                                    |
| Fosfomycin                | 200 µg       | CLSI     | 22-30  | 25-33                          |                                    |                                    |                                    |
| Fusidic acid              | 10 µg        | CA-SFM   |  | 29-34                          |                                    |                                    |                                    |
| Gentamicin                | 10 µg        | CLSI     | 19-26  | 19-27                          | 16-21                              |                                    |                                    |
| Gentamicin                | 10 UI        | CA-SFM   | 22-26  | 24-28                          | 16-22                              |                                    |                                    |
| Imipenem                  | 10 µg        | CLSI     | 26-32  |                                | 20-28                              |                                    |                                    |
| Isepamicin                | 30 µg        | Internal | 21-26  | 17-23                          | 16-23                              |                                    |                                    |
| Kanamycin                 | 30 µg        | CLSI     | 17-25  | 19-26                          |                                    |                                    |                                    |
| Levofloxacin              | 5 µg         | CLSI     | 29-37  | 25-30                          | 19-26                              |                                    |                                    |
| Lincomycin                | 15 µg        | CA-SFM   |  | 25-29                          |                                    |                                    |                                    |
| Linezolid                 | 30 µg        | CLSI     |  | 25-32                          |                                    |                                    |                                    |
| Mecillinam                | 10 µg        | CLSI     | 24-30  |                                |                                    |                                    |                                    |
| Meropenem                 | 10 µg        | CLSI     | 28-34  | 29-37                          | 27-33                              |                                    |                                    |
| Mezlocillin               | 75 µg        | CLSI     | 23-29  |                                | 19-25                              |                                    |                                    |
| Minocycline               | 30 µg        | CLSI     | 19-25  | 25-30                          |                                    |                                    |                                    |
| Moxalactam                | 30 µg        | CLSI     | 28-35  | 18-24                          | 17-25                              |                                    |                                    |
| Moxifloxacin              | 5 µg         | CLSI     | 28-35  | 28-35                          | 17-25                              |                                    |                                    |
| Mupirocin                 | 5 µg         | Internal |  | 18-26                          |                                    |                                    |                                    |
| Nalidixic Acid            | 30 µg        | CLSI     | 22-28  |                                |                                    |                                    |                                    |
| Neomycin                  | 30 UI        | Internal | 17-23  | 18-27                          |                                    |                                    |                                    |
| Netilmicin                | 30 µg        | CLSI     | 22-30  | 22-31                          | 17-23                              |                                    |                                    |
| Nitrofurantoin            | 300 µg       | CLSI     | 20-25  | 18-22                          |                                    |                                    |                                    |
| Nitroxolin                | 20 µg        | Internal | 15-22  |                                |                                    |                                    |                                    |
| Norfloxacin               | 5 µg         | Internal | 26-33  | 13-23                          | 20-27                              |                                    |                                    |
| Norfloxacin               | 10 µg        | CLSI     | 28-35  | 17-28                          | 22-29                              |                                    |                                    |
| Ofloxacin                 | 5 µg         | Internal | 25-32  | 21-28                          | 14-20                              |                                    |                                    |
| Oxacillin                 | 5 µg         | CA-SFM   |  | 27-34                          |                                    |                                    |                                    |
| Oxacillin                 | 1 µg         | CLSI     |  | 18-24                          |                                    |                                    |                                    |
| Oxolinic Acid             | 10 µg        | Internal | 22-30  |                                |                                    |                                    |                                    |
| Pefloxacin                | 5 µg         | CA-SFM   | 29-35  | 26-29                          |                                    |                                    |                                    |
| Penicillin                | 6 µg         | CLSI     |  | 26-37                          |                                    |                                    |                                    |
| Pipemidic Acid            | 20 µg        | Internal | 22-30  |                                |                                    |                                    |                                    |
| Piperacillin              | 100 µg       | CLSI     | 24-30  |                                | 25-33                              |                                    |                                    |
| Piperacillin              | 75 µg        | Internal |  |                                | 24-33                              |                                    |                                    |
| Piperacillin + Tazobactam | 75/10 µg     | Internal | 22-29  | 24-31                          | 24-33                              |                                    |                                    |
| Piperacillin + Tazobactam | 100/10 µg    | CLSI     | 24-30  | 27-36                          | 25-33                              |                                    |                                    |
| Polymixin                 | 300 UI       | CLSI     | 13-19  |                                |                                    |                                    |                                    |
| Pristinamycin             | 15 µg        | CA-SFM   |  | 27-32                          |                                    |                                    |                                    |
| Quinupristin-Dalfopristin | 15 µg        | CLSI     |  | 21-28                          |                                    | 19-24                              | 15-21                              |
| Rifampicin                | 5 µg         | CLSI     |  | 26-34                          |                                    |                                    |                                    |
| Rifampicin                | 30 µg        | CA-SFM   |  | 34-39                          |                                    |                                    |                                    |
| Sparfloxacin              | 5 µg         | CLSI     | 30-38  | 27-33                          | 21-29                              |                                    |                                    |
| Spectinomycin             | 100 µg       | Internal | 19-25  |                                |                                    |                                    |                                    |
| Spiramycin                | 100 µg       | Internal |  | 18-26                          |                                    |                                    |                                    |
| Streptomycin              | 10 µg        | CLSI     | 12-20  | 14-22                          |                                    |                                    |                                    |
| Sulfonamides              | 300 µg       | CLSI     |  | 24-34                          |                                    |                                    |                                    |
| Sulfonamides              | 200 µg       | Internal |  | 17-27                          |                                    |                                    |                                    |
| Teicoplanin               | 30 µg        | CLSI     |  | 15-21                          |                                    |                                    |                                    |
| Telithromycin             | 15 µg        | CLSI     |  |                                |                                    | 27-33                              | 17-23                              |
| Tetracycline              | 30 µg        | CLSI     | 18-25  | 24-30                          |                                    |                                    |                                    |

|                                 | Disk Content  | Standard | Acceptable Zone Diameter (mm) Quality Control Limits |                             |                                 |                                 |                                 |
|---------------------------------|---------------|----------|--|-----------------------------|---------------------------------|---------------------------------|---------------------------------|
|                                 |               |          | <i>E. coli</i> ATCC 25922                            | <i>S. aureus</i> ATCC 25923 | <i>P. aeruginosa</i> ATCC 27853 | <i>S. pneumoniae</i> ATCC 49619 | <i>H. influenzae</i> ATCC 49247 |
| Ticarcillin                     | 75 µg         | CLSI     | 24-30  |                             | 21-27                           |                                 |                                 |
| Ticarcillin + Clavulanic Acid   | 75/10 µg      | CLSI     | 24-30  | 29-37                       | 20-28                           |                                 |                                 |
| Tigecycline                     | 15 µg         | CLSI     | 20-27  | 20-25                       |                                 | 23-29                           |                                 |
| Tobramycin                      | 10 µg         | CLSI     | 18-26  | 19-29                       | 19-25                           |                                 |                                 |
| Trimethoprim                    | 5 µg          | CLSI     | 21-28  | 19-26                       |                                 |                                 |                                 |
| Trimethoprim + Sulfamethoxazole | 1,25/23,75 µg | CLSI     | 23-29  | 24-32                       |                                 |                                 |                                 |
| Vancomycin                      | 30 µg         | CLSI     |  | 17-21                       |                                 |                                 |                                 |

**For control limits (Internal standards or CLSI):**

- Gentamicin 120 µg : *E. faecalis* ATCC 29212 (16-23 mm)
- Streptomycin 300 µg : *E. faecalis* ATCC 29212 (14-20 mm)
- Gentamicin 500 µg : *S. aureus* ATCC 25923 (30-34 mm) ; *E. faecalis* ATCC 29212 (18 -28 mm)
- Kanamycin 1mg : *S. aureus* ATCC 25923 ( $\geq 14$  mm) ; *E. faecalis* ATCC 29212 ( $\leq 10$  mm).
- Streptomycin 500 µg : *S. aureus* ATCC 25923 (24-28 mm) ; *E. faecalis* ATCC 29212 (14-25 mm).
- Metronidazole 4µg : *Clostridium perfringens* ATCC 13124, *Clostridium sporogenes* ATCC 19404  $\geq 15$  mm
- Cefoperazone + Sulbactam 75 / 30 µg: *E. coli* ATCC 35218 (24-30 mm)

*Haemophilus influenzae* ATCC 10211 is recommended as a useful additional quality control strain to verify the growth promotion properties of HTM.

Despite the lack of reliable disk diffusion interpretative criteria for *S.pneumoniae* with certain Beta-lactams, *Streptococcus pneumoniae* ATCC 49619 is the strain designated for quality control of all disks diffusion tests with all *Streptococcus* spp.

*Neisseria gonorrhoeae* ATCC 49226 is recommended to test spectinomycin disk with acceptable diameters of 23 to 29 mm (CLSI). Deterioration in oxacillin 1µg disk content is best assessed with QC organism *Staphylococcus aureus* ATCC 25923, with acceptable diameter of 18 to 24 mm.

Following the EUCAST guidelines (version1.2, 2010), the performances of antibiotic disks are systematically controlled by using the following strains:

- *Escherichia coli* ATCC 25922
- *Pseudomonas aeruginosa* ATCC 27853
- *Enterococcus faecalis* ATCC 29212
- *Staphylococcus aureus* ATCC 29213
- *Streptococcus pneumoniae* ATCC 49619
- *Haemophilus influenzae* NCTC 8468

The table 3 indicates the accepted limits for the diameters of inhibition obtained by the agar diffusion method for the reference strains indicated below.

|                           | Disk Content | Acceptable Zone Diameter (mm) Quality Control Limits |                             |                                 |                                 |                                |                               |
|---------------------------|--------------|--|-----------------------------|---------------------------------|---------------------------------|--------------------------------|-------------------------------|
|                           |              | <i>E. coli</i> ATCC 25922                            | <i>S. aureus</i> ATCC 29213 | <i>P. aeruginosa</i> ATCC 27853 | <i>S. pneumoniae</i> ATCC 49619 | <i>H. influenzae</i> NCTC 8468 | <i>E. faecalis</i> ATCC 29212 |
| Ampicillin                | 2 µg         |  |                             |                                 |                                 | 19-25                          | 15-21                         |
| Cefotaxime                | 5 µg         | 25-31  |                             |                                 |                                 | 29-35                          |                               |
| Ceftazidime               | 10 µg        | 23-29  |                             | 21-27                           |                                 |                                |                               |
| Doripenem                 | 10 µg        | 27-35  |                             | 28-35                           | 31-37                           |                                |                               |
| Gentamicin                | 30 µg        |  |                             |                                 |                                 |                                | 12-18                         |
| Linezolid                 | 10 µg        |  | 21-27                       |                                 | 23-29                           |                                | 19-25                         |
| Netilmicin                | 10 µg        | 18-24  | 20-26                       | 15-21                           |                                 |                                |                               |
| Nitrofurantoin            | 100 µg       |  | 17-23                       |                                 |                                 |                                | 18-24                         |
| Penicillin                | 1 IU         |  | 12-18                       |                                 |                                 |                                |                               |
| Piperacillin              | 30 µg        | 21-27  |                             |                                 |                                 |                                |                               |
| Piperacillin + Tazobactam | 30/6 µg      |  |                             | 23-29                           |                                 |                                | 20-26                         |
| Tobramycin                | 30 µg        |  |                             |                                 |                                 |                                | 12-18                         |
| Vancomycin                | 5 µg         |  |                             |                                 | 17-23                           |                                | 10-16                         |

**10- QUALITY CONTROL OF THE MANUFACTURER**

All manufactured and commercialized reagents are under complete quality system starting from reception of raw material to the final commercialization of the product.

Each lot is submitted to a quality control and only is released on the market when conforming to the acceptance criteria.

The records relating to production and control of each single lot are kept within our company

## 11- LIMITATIONS OF USE

- Clinical categorisations (resistant, intermediate, susceptible...) vary by only one millimeter, which corresponds to a common margin of error in the laboratory.
- Antimicrobial agents other than indicated in table 2 may be used. Susceptibility tests employing these agents should be interpreted on the basis of presence or absence of a definite zone of inhibition and should be considered as only qualitative until such time as interpretative zones have been established. All zone diameters should be recorded.
- The performances of the test depend not only on the activity of the disks, but also on factors such as the use of an appropriate inoculum and control strains, appropriate and previously tested culture media, an adequate storage.
- The final interpretation, as for all laboratory interpretations, can not be based on the results of one single test but on an overview of the clinical data and the biochemical, cytological and immunological results.

## 12- REFERENCES

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6. V. Loncle-Provot, E. Keller, M.O. Gourdin, M.L. Garrigues. Etude de la stabilité des disques antibiotiques dans les conditions d'utilisation en routine, 18th inter disciplinary meeting on anti-infectious chemotherapy, Paris, Dec.3/4 1998.



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