# EUCAST-and CLSI potency
# NEO-SENSITABS™



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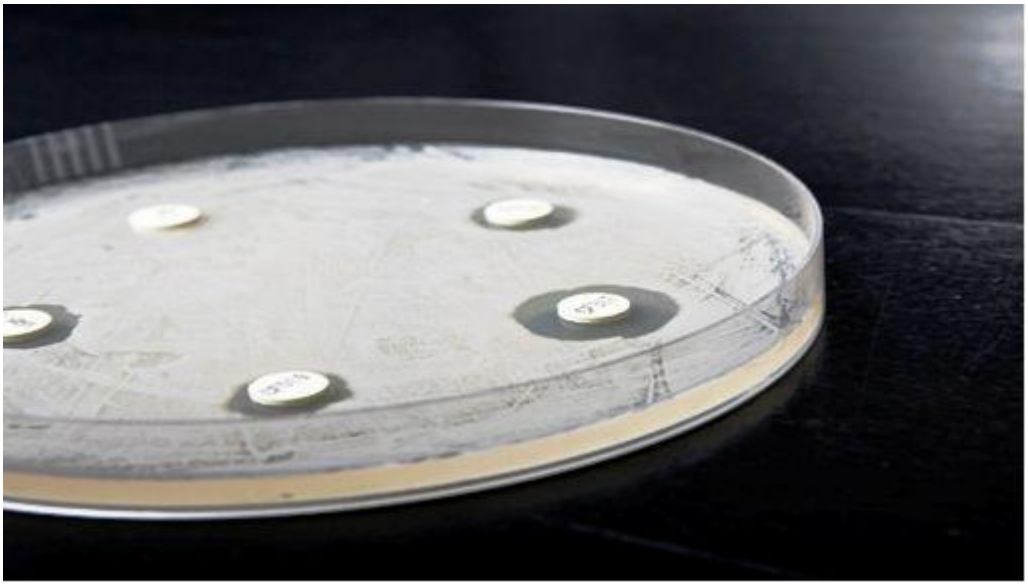
# NEO-SENSITABS™

# User's Guide

## Susceptibility testing



## EUCAST and CLSI potency
## Neo-Sensitabs™

**2013**

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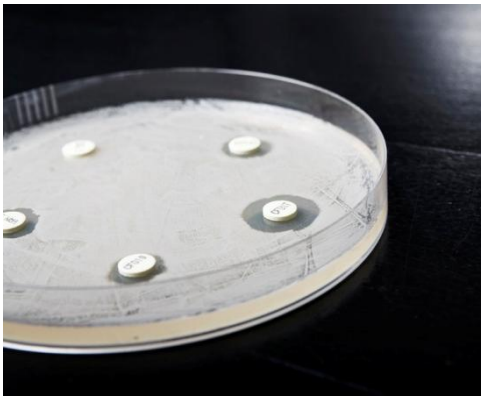
# EUCAST and CLSI-potency Neo-Sensitabs™ User's Guide 2013



EUCAST and CLSI-potency NEO-SENSITABS™ User's Guide 2013 contains updated text, tables and references, all necessary information when using Neo-Sensitabs tablets for susceptibility testing.

Totally new interpretation tables using Mueller Hinton Agar and McFarland 0.5 inoculum according to the MIC breakpoints recommended by EUCAST are included.

The different interpretation tables following the CLSI (formerly NCCLS) recommendations have been updated according to the latest information of the CLSI described in "Performance Standards for Antimicrobial Disk Susceptibility Testing", 23rd Informational Suppl., **M100-S23**, 2013.

Furthermore, the User's Guide includes updated Zone Diameter Interpretative Standards according to national recommendation groups including interpretation according to MIC breakpoints recommended by the SFM and BSAC has been updated.

Other new aspects that have been revised in the updated User's Guide of the EUCAST and CLSI-potency NEO-SENSITABS™ are:

- 2 + 18 hours (or 2+22) hours prediffusion method for high molecular weight antimicrobials: Colistin, Daptomycin, Vancomycin and Teicoplanin has been included.
- Interpretative zone breakpoints for 2+18 hours' prediffusion method for Daptomycin, Vancomycin and Teicoplanin in the different tables.
- Prediffusion method with Colistin is recommended for both *A. baumannii* and *P. aeruginosa* strains.
- Kits for detection of ESBLs using Neo-Sensitabs have been developed.
- Kit for detecting Plasmid-mediated AmpC beta-lactamases, using Cefotaxime, Ceftazidime and Cloxacillin has been developed.
- Kits for detecting carbapenemases class A (KPC and GES enzymes), class D (metallo-betalactamases) and OXA-48 have been developed.
- Kit for detecting metallo-ß-lactamases using 2 chelating agents EDTA and Dipicolinic acid has been developed.
- New drugs that will be introduced in the near future are: Ceftazidime+Avibactam, Ceftaroline+Avibactam, Ceftolozane+Tazobactam, CXA-101, Tedizolid (TR-700), Monosulfactam (BAL30072), Fidaxomicin (OPT-80), Solithromycin (CEM-101), Deformylase inhibitor and new Fluoroquinolones.

The User's Guide for EUCAST and CLSI-potency Neo-Sensitabs™ 2013, is available on our website www.rosco.dk and updated information is continuously included.

ROSCO Diagnostica A/S is welcoming any feedback and questions on susceptibility testing from users directly (info@rosco.dk) or through our representatives.

**ROSCO Diagnostica A/S**

Characteristics of Neo-Sensitabs

Neo-Sensitabs are 9 mm in diameter and each is print coded for safe identification. The tablets are manufactured with the aid of microbial inert auxiliary substances by a dry process using crystalline antimicrobials. This procedure results in very uniform tablets that are homogenous in their content of active ingredients and have an extraordinary stability, usually not less than 4 years shelf-life at room temperature (chapter 1).

Neo-Sensitabs and the MIC-breakpoints (chapter 3) are standardized according to potencies recommended by CLSI- Clinical and Laboratory Standards Institute (formerly NCCLS, EUCAST, SFM France (chapter 5) and BSAC (chapter 6). The size of the zone therefore is equivalent to the size of a paper disk, if the potency is the same.

Recently EUCAST (chapter 4) informed that they will develop a disk diffusion method on Mueller-Hinton agar. Neo-Sensitabs will follow the EUCAST recommendations.

All antimicrobials have received letter codes in order to achieve optimal recognition and zone measurements with automatic instruments a 5-digit code for each Neo-Sensitabs type has been chosen. The new Neo-Sensitabs with potency according to CLSI have of course different code than the old Neo-Sensitabs e.g. Erythromycin 78 µg (code: ERYTR) and Erythromycin 15 µg (code: ERY15).

The new ranges of Neo-Sensitabs are produced in cartridges with spring and are used together with the new dispenser from Rosco. The interpretation tables are available in this supplement; however, some additional information concerning Neo-Sensitabs is still only available in the User's Guide for Neo-Sensitabs Ed. 19, 2007/2008 (soon Ed. 20, 2009).

Dispenser

The dispensers are to be used with Rosco Neo-Sensitabs **cartridges with a spring**, where the potencies of the tablets are according to recommendations of CLSI (formerly NCCLS).

Models available from Rosco:

1) Adaptable to 8-10 cm petri dishes delivering up to 7 Neo-Sensitabs at a time (Dispenser 101).
2) Adaptable for square petri dishes (12 x 12 cm) delivering up to 16 Neo-Sensitabs at a time (Dispenser 104).
3) A single cartridge dispenser.

The Dispensers are made of hard plastic and are operated by pushing the handle down, and the Neo-Sensitabs will be transferred to the agar surface.
When using several dispensers, the colour code on the top of the handles can be used for differentiation.
The holes in the bottom plate ensure that the tablets are placed onto the agar surface in a pre-determined pattern.
The dispensers are easy to disassemble for inside cleaning. They must be cleaned occasionally (wipe with ethanol and hot water to remove dust from the Neo-Sensitabs tablets).

The tablets come packed in cartridges (tubes), matching the dispenser top-plate holes. Each cartridge accommodates 50 Neo-Sensitabs and a red block that prevents dispensing when one cartridge is empty. Insert the cartridges gently and carefully one by one through the top-plates holes. For easy identification the bottom of each tube is labeled with a short name (5-digit alpha-numeric code, also marked on each tablet) of the antimicrobial contained in the cartridge.

Further information is available on www.rosco.dk

**Storage instructions**

1. On receipt check the temperature symbol on the outer container.
Neo-Sensitabs with a 2 °C to 8 °C symbol should be stored in a refrigerator and Neo-Sensitabs with a 25 °C as an upper temperature symbol on the outer container should be stored at room temperature (≤25°C).

2. If Neo-Sensitabs are stored in the refrigerator, allow the cartridge to reach room temperature (≤25°C) before being opened, i.e. 30 – 60 minutes, in order to avoid water condensation on the tablets.

3. Neo-Sensitabs with the temperature symbol 2 °C to 8 °C may be left at room temperature (≤25°C) for up to 2 months, without essential loss of activity.

4. Opened cartridges placed in a dispenser must be used within 2 months for Neo-Sensitabs with the temperature symbol 2 °C to 8 °C, and within 12 months for Neo-Sensitabs with the temperature symbol below 25 °C. The dispenser must be kept at room temperature.

The stability of antimicrobials in paper disks is decreased compared to Neo-Sensitabs. The CLSI (1) recommends frozen storage of paper disks containing beta-lactam antibiotics. In case of Imipenem, Cefaclor and Clavulanic acid combinations, paper disks should be stored frozen until the day of use.
In a comparative stability study between Neo-Sensitabs and Oxoid paper disks (2), it was observed that disks containing Ticarcillin 75 µg , lost activity after 15 days at 4-6 °C, while Ampicillin 10 µg and Amoxycillin + Clavulanate 20+10 µg disks lost activity after one month at 4-6 °C.
The corresponding Neo-Sensitabs were stable at room temperature (and at 4-6 °C) during the study period of six months.

Steward et al (3) noticed overdetection of imipenem/meropenem resistance in the project ICARE, most probably due to inactivation of the reagents used (Vitek, disk diffusion etc.) and recommended the use of a second independent antimicrobial susceptibility testing method to validate carbapenemintermediate and resistant strains.

**References:**
1) NCCLS: Performance Standards for Antimicrobial Disk Susceptibility Testing, 10th Ed. **M2-A10**, January 2009
2) del Cuerpo M. et al: Stability of beta-lactam antibiotics in paper disks and tablets used in the diffusion test. Rev. Esp. Quimioter., **10**, nr. 3,1997 (Spanish).
3) Steward C.D. et al: Antimicrobial susceptibility testing of carbepenems: multicenter validity testing and accuracy levels of 5 antimicrobial test methods for detecting resistance in Enterobacteriaceae and Pseudomonas aeruginosa isolates. J. Clin. Microbiol., **41**, 351-358, 2003.

Inoculum

When using the technique of Kirby-Bauer, the inoculum is standardized according to the method described by the CLSI (chapter 2 and 3), which results in confluent growth for bacteriae and semi-confluent growth for most of the *Candida* species isolates.

Inoculum standardisation

**Direct colony suspension method:**
Suspend several morphologically similar colonies from an 18-24 h agar plate (non selective) into 4-5 ml sterile saline solution, and then immediately adjusting the turbidity to match that of the $BaSO_4$ standard (0.5 McFarland). For *Candida* spp. that are subcultured onto blood agar or Sabouraud dextrose agar five distinct colonies from a 24-hour-old culture are suspended in 5 ml sterile saline (0.145 mol/L; 8.5 g/L NaCl; 0.85% saline).

a) Within 15 minutes, dip a sterile cotton swab into the adjusted suspension and remove inoculum from the swab by exerting firm pressure on the inside of the tube.
b) Within 15 minutes swabs are used to inoculate the test plates.
c) Inoculate the dried surface of the appropriate agar plate by streaking the swab over the entire surface. Allow the surface to dry 3-5 minutes or maximum 15 minutes before applying Neo-Sensitabs to the media.
d) Select appropriate tablets e.g. such as recommended by CLSI (NCCLS).[2] Use no more than nine Neo-Sensitabs per 150 mm plate or four Neo-Sensitabs per 100 mm plate when testing *Candida* spp., *H. influenzae, N. gonorrhoeae,* and *Streptococcus* spp.
e) Neo-Sensitabs is dispensed onto the surface of the inoculated agar plate.

Incubation and reading of plates

a) Within 15 minutes, place the agar side up in a 35 °C (± 2 °C) incubator. For some strains special recommendations are noted in the tables e.g. *Haemophilus* spp., *N. gonorrhoeae*, *S. pneumoniae* and other streptococci should be incubated in an atmosphere enriched with 5 % $CO_2$.
Incubate no more than 5 plates in a stack. Plates in the middle of the stack will take longer to reach the desired incubation temperature than plates at the top and the bottom.

b) Examine the plates after 16-18 hours incubation (20-24 h for *Candida* spp. *N. gonorrhoeae*, *S. pneumoniae* and other streptococci). Full 24-hour incubation is recommended for the detection of Methicillin resistant *Staphylococcus aureus* (MRSA) and *Enterococcus* spp. for vancomycin resistance. Hold the plate up to transmitted light and examine the oxacillin, linezolid and vancomycin zones for light growth (minute colonies) of methicillin, linezolid or vancomycin resistant colonies, respectively, within apparent zones of inhibition. Any discernible growth within the zone of inhibition is indicative of methicillin, linezolid or vancomycin-resistance. The edges of the zones of inhibition contain a large number of small colonies when using Trimethoprim, Sulphonamides and Trimethoprim + Sulfamethoxazole tablets. In this case zones of inhibition are measured up to colonies of normal size (disregard slight growth and measure the more obvious margin).

c) For some antimicrobial agents (chloramphenicol, clindamycin, erythromycin and tetracycline) the zones of inhibition will contain a gradient of growth. In this case zone diameters should be read half-way between the start of inhibition and complete inhibition.

d) With *Proteus* spp. ignore the thin veil of swarming growth in an otherwise obvious zone of inhibition. The diameters of the zones of complete inhibition are measured as determined by gross visual inspection. Zones are measured to the nearest whole millimeter.

e) The measured zone diameters of inhibition are compared with the zone interpretative tables for the individual antibiotics in order to determine the agent(s) most suitable for use in antimicrobial therapy.

**Pre-diffusion Method (2 + 18 or 2+22 hours) for Antimicrobials Diffusing Poorly on Agar**

High molecular weight antimicrobials (Vancomycin, Teicoplanin, Daptomycin, Colistin) diffuse poorly on agar media, resulting in difficult to interpret results when using the disc diffusion method. Rosco Diagnostica has developed a 2 + 18 or 2 + 22 hour prediffusion technique, permitting an easier differentiation between susceptible and resistant strains when testing against these antimicrobials.

**Procedure**

One Neo-Sensitabs of the antimicrobial to be tested is placed on an uninoculated plate containing the susceptibility test medium (Mueller-Hinton plain or BHI Agar + 5 % blood).
The plate is then placed in the incubator at 37°C for 2 hours. After 2 hours the tablet (disc) is removed (by knocking the plate against the table) and the plate is maintained at room temperature for further 18 or 22 hours (overnight).

The plate is now inoculated with the strain to be tested using a McFarland 0.5 inoculum.

Additional antibiotic discs (Neo-Sensitabs) may be added now using a dispenser (if MH agar is used) and thereafter the plate is incubated overnight at 35-37 °C.

The zones of inhibition are then measured. Zone breakpoints are tentative and for research use only.

**Notice**: In the laboratory, the prediffusion plate can be prepared the day before it is inoculated, in which case there is no loss of time and results are obtained within 24 hours.

**Interpretation**

IA) Detection of Visa/GISA/hVISA strains (medium BHI + 5 % blood or MH plain

VISA/GISA/hVISA strains will show the following zones of inhibition:

| BHI+5% blood | | MH plain |
|---|---|---|
| Teicoplanin 30 µg: inhibition zone < 20 mm | and/or | Teicoplanin R< 20 mm |
| Vancomycin 30 µg: inhibition zone < 20 mm | | Vancomycin R≤ 20 mm |

IB) Detection of GISCN/hGISCH strains (medium BHI + 5 % blod)

Teicoplanin 30 µg: inhibition zone < 20 mm

GISCN = Glycopeptide intermediate staphylococci, coagulase negative (2).

Strains showing zones of inhibition < 20 mm around Teicoplanin 30 µg should be reported as heteroresistant to both Teicoplanin and Vancomycin.

IC) Detection of VanA, VanB and VanC in enterococci

Use Mueller-Hinton Agar (without blood add), McFarland 0.5 inoculum.

Vancomycin 30 µg (2+18 hours' prediffusion method):

| | |
|---|---|
| Susceptible: | zone > 16 mm (sharp edge) |
| VanB: | zone < 16 mm (hazy edge) |
| VanC: | zone < 12mm (sharp edge) |

The VanA genotype will show **no zone** of inhibition in the current difffusion test with Vancomycin 30 µg Neo-Sensitabs.

ID) Detection of VRE,vanB phenotype and vanA genotype

Use Mueller-Hinton Agar,Mc Farland 0.5 inoculum

Vancomycin 30 ug : no zone

Teicoplanin 30 ug : zone < 16 mm (MIC 4-12 ug/ml). Report as R

II) Daptomycin testing (medium used Mueller-Hinton plain)

a) Staphylococci:

Daptomycin 30 µg Neo-Sensitabs: ≤ 1 µg/ml). Susceptible zone ≥ 22 mm (corresponding to an MIC of Resistant zone <20 mm (MIC ≥ 2 µg/ml)

b) Enterococci:

Daptomycin 30 µg Neo-Sensitabs: ≤ 4 µg/ml). Susceptible zone ≥12 mm (corresponding to an MIC of

III) Colistin testing (medium used Mueller-Hinton plain)

Colistin 10 µg Neo-Sensitabs: Susceptible zone ≥15 mm (corresponding to an MIC of ≤ 2 µg/ml).

**References:**

1) Nielsen S.V., Casals J.B.: Detection of decreased susceptibility to glycopeptides in *S. aureus* using tablet (disc) prediffusion. 15th Eur. Cong. Clin. Microbiol. Inf. Dis. (ECCMID), April 2005.
2) Ferreira Nunes AP et al: Heterogeneous resistance to vancomycin in S. epidermidis, S. haemolyticus and S. warneri clinical strains: characterisation of glycopeptide susceptibility profiles and cell wall thickening. Intl. J. Antimicr. Ag., **27**, 307-315, 2006.
3) Katz B.D. et L: A new pre-diffusion method for the detection of Daptomycin (DAP) non-susceptible strains using Neo-Sensitabs. Presentation D-226, ICAAC September 2007, Chicago, USA.
4) Borda N. et al: Comparison of methods: diffusion (DF), prediffusion (PDF) and E-test on isolates of Ac. baumannii-calcoaceticus complex (Abc) against colistin. 2007 (in press).
5) Katz B.D. et al: Detection of daptomycin- non-susceptible strains using Neo-Sensitabs trade pre-diffusion method. Diagn. Microbiol. Infect. Dis. **61**. 315-320, 2008.
6) Koeth L. et al: Multisite evaluation of the Daptomycin Neo-Sensitab prediffusion method against 20 S. aureus. ECCMID, Milan 2011.
7) Boyen F. et al: Disk prediffusion is a reliable method for testing Colistin susceptibility in porcine E. coli strains. Vet. Microbiol. **144**, 359-362, 2010.

**Note:**

Detailed description of the prediffusion methods for Vancomycin, Teicoplanin, Daptomycin and Colistin see: "Detection of resistance mechanisms using Neo-Sensitabs and Diatabs" documents 7.1.0; 7.2.0; 7.3.0; 7.4.0 and 7.5.0.

**Susceptible (S):**
The infection due to the strain tested may be expected to respond to a normal dosage of this antimicrobial.

**Intermediate (I)**
The intermediate category implies clinical applicability in body sites where the drugs are concentrated (e.g. urine) or when high dosage of an antimicrobial can be used (e.g. betalactams).

The intermediate category also comprises a "buffer zone" which should prevent small uncontrolled technical factors from causing major discrepancies in interpretations; thus, when a zone falls within the intermediate range, the results may be considered equivocal, and if alternative drugs are not available MIC testing may be indicated.

**Resistant (R):**
The antimicrobial cannot be recommended for treatment in this case.

If only "S" criteria are specified:
For some organism/antimicrobial combinations, the absence of resistant strains precludes defining any category other than susceptible. For strains yielding results suggestive of "non susceptible", organism identification and antimicrobial susceptibility test results should be confirmed. Subsequently the isolates should be submitted to a Reference Laboratory for further testing.

**References:**
1) Gylling Pedersen O.: Standardizing, manufacture, and control of Neo-Sensitabs. Acta Clin. Belg., **28**, 139-149, 1973.
2) CLSI: Performance Standards for Antimicrobial Susceptibility Testing 20th Inf. Suppl. **M100-S20**, 2010.
3) NCCLS: Performance Standards for Antimicrobial Disk Susceptibility Testing. 8th Ed. **M2-A9,** 2006.
4) NCCLS: Method for Antifungal Disk Diffusion Susceptibility Testing. **M44-A2**, 2008.
5) Kauppila J. et al: Comparison of Neo-Sensitabs with paper disks in the routine disk diffusion antimicrobial susceptibility testing. ECCMID poster P-865, Barcelona 2008.
6) Kahlmeter G: Implementation of European breakpoints and the future of EUCAST. ECCMID abstract 512, april 2008, Barcelona.

**Antibacterials**

| Neo-Sensitabs | Identification Code Neo-Sensitabs | Diffusible Amount of Antimicrobial |
|---|---|---|
| **A. Penicillins (Penams)** | | |
| PENICILLIN 1 UNIT EUCAST | PENG1 | 1 U |
| PENICILLIN | PEN10 | 10 U |
| AMPICILLIN | AMP10 | 10 µg |
| AMOXYCILLIN 25 µg BSAC (U) | AMX25 | 25 µg |
| AMOXYCILLIN | AMOXY | 30 µg |
| AMPICILLIN 2 µg EUCAST | AMP.2 | 2 µg |
| OXACILLIN 1 µg | OXA.1 | 1 µg |
| MECILLINAM (Amdinocillin) | MEC10 | 10 µg |
| TICARCILLIN | TIC75 | 75 µg |
| PIPERACILLIN | PIPER | 100 µg |
| PIPERACILLIN EUCAST | PIP30 | 30 µg |
| TEMOCILLIN | TEMOC | 30 µg |
| **B. Beta-lactam / Beta-lactamase Inhibitor Combinations** | | |
| AMOXYCILLIN+CLAVULANATE 20+10 µg | AMC30 | 20+10 µg |
| AMOXYCILLIN+CLAVULANATE 2+1 µg (Augmentin) | AMC.3 | 1+2 µg |
| AMPICILLIN+SULBACTAM | SAM20 | 10+10 µg |
| TICARCILLIN+CLAVULANATE (Timentin) | TIM85 | 75+10 µg |
| PIPERACILLIN+TAZOBACTAM | PI+TZ | 100+10 µg |
| PIPERACILLIN+TAZOBACTAM BSAC | PTZ85 | 75+10 µg |
| CEFOTAXIME+CLAVULANATE | CTX+C | 30+10 µg |
| CEFTAZIDIME+CLAVULANATE | CAZ+C | 30+10 µg |
| CEFEPIME+CLAVULANATE | FEP+C | 30+10 µg |
| CEFPODOXIME+CLAVULANATE* | CPD+C | 10+1 µg |
| PIPERACILLIN +TAZOBACTAM EUCAST | PTZ36 | 30+6 µg |
| **C. (1) Cephalosporins (Cephems)** | | |
| CEPHALOTHIN | CEP30 | 30 µg |
| CEFACLOR | CCL30 | 30 µg |
| CEFAZOLIN | CFZ30 | 30 µg |
| CEPHALEXIN BSAC (U) | CFLEX | 30 µg |
| CEFADROXIL | CDX30 | 30 µg |
| CEFUROXIME | CXM30 | 30 µg |
| CEFIXIME | CFM.5 | 5 µg |
| CEFPODOXIME | CPD10 | 10 µg |
| CEFOTAXIME | CTX30 | 30 µg |
| CEFTAZIDIME | CAZ30 | 30 µg |
| CEFTRIAXONE | CTR30 | 30 µg |
| CEFTIZOXIME | ZOX30 | 30 µg |
| CEFEPIME | FEP30 | 30 µg |
| CEFOTAXIME EUCAST | CTX.5 | 5 µg |
| CEFTAZIDIME EUCAST | CAZ10 | 10 µg |
| CEFTIBUTEN EUCAST | CTB30 | 30 µg |
| **C. (2) Cephamycins and Oxacephems** | | |
| CEFOXITIN | CFO30 | 30 µg |
| CEFOXITIN** | CFO10 | 10 µg |

| Neo-Sensitabs | Identification Code Neo-Sensitabs | Diffusible Amount of Antimicrobial |
|---|---|---|
| **C. (3) Cephalosporins active against MRSA** | | |
| CEFTOBIPROLE (investigational drug) | CFBIP | 30 µg |
| CEFTAROLINE (investigational drug) | CPT30 | 30 µg |
| **D. Penems and Carbapenems** | | |
| IMIPENEM | IMI10 | 10 µg |
| MEROPENEM | MRP10 | 10 µg |
| ERTAPENEM | ETP10 | 10 µg |
| DORIPENEM | DOR10 | 10 µg |
| **E. Monobactams** | | |
| AZTREONAM | AZT30 | 30 µg |
| **F. Aminoglycosides** | | |
| STREPTOMYCINS 10 µg | STR10 | 10 µg |
| STREPTOMYCIN 500 µg (HLR)** | ST500 | 500 µg |
| KANAMYCIN 30 µg | KAN30 | 30 µg |
| KANAMYCIN 500 µg (HLR)** | KA500 | 500 µg |
| NEOMYCIN (Framycetin)* | NEOMY | 120 µg |
| AMIKACIN | AMI30 | 30 µg |
| GENTAMICIN 10 µg | GEN10 | 10 µg |
| GENTAMICIN 30 µg EUCAST | GEN30 | 30 µg |
| GENTAMICIN 250 µg (HLR)** | GN250 | 250 µg |
| NETILMICIN 10 µg EUCAST | NET10 | 10 µg |
| NETILMICIN 30 µg | NET30 | 30 µg |
| TOBRAMYCIN | TOB10 | 10 µg |
| SPECTINOMYCIN | SPECT | 200 µg |
| **G. Tetracyclines** | | |
| TETRACYCLINES 30 µg (Oxytetracycline) | TET30 | 30 µg |
| DOXYCYCLINE | DOX30 | 30 µg |
| MINOCYCLINE | MIN30 | 30 µg |
| TIGECYCLINE | TIG15 | 15 µg |
| **H. Chloramphenicol and Derivatives** | | |
| CHLORAMPHENICOL 30 µg | CLR30 | 30 µg |
| **I. Macrolides, Lincosamides, Streptogramines, Ketolides and Oxazolidinones** | | |
| ERYTHROMYCIN | ERY15 | 15 µg |
| AZITHROMYCIN | AZI15 | 15 µg |
| CLARITHROMYCIN | CLA15 | 15 µg |
| CLINDAMYCIN | CLI.2 | 2 µg |
| QUINUPRISTIN/DALFOPRISTIN | SYN15 | 15 µg |
| TELITHROMYCIN | TEL15 | 15 µg |
| LINEZOLID | LINEZ | 30 µg |
| LINEZOLID EUCAST | LIZ10 | 10 µg |
| **J. (1) Glycopeptides** | | |
| VANCOMYCIN 5 µg EUCAST | VAN.5 | 5 µg |
| VANCOMYCIN 30 µg | VAN30 | 30 µg |
| **J. (2) Lipoglycopeptides** | | |
| TELAVANCIN (investigational drug) | TLV30 | 30 µg |
| TEICOPLANIN | TPN30 | 30 µg |

| Neo-Sensitabs | Identification Code Neo-Sensitabs | Diffusible Amount of Antimicrobial |
|---|---|---|
| DALBAVANCIN (investigational drug) | DAL60 | 60 µg |
| **J. (3) Cyclic Lipopeptides** | | |
| a) Gram positive spectrum: | | |
| DAPTOMYCIN (+ Ca) | DAPCa | 30 µg |
| b) Gram negative spectrum: | | |
| COLISTIN 10 µg | CO.10 | 10 µg |
| POLYMYXINS (not for susceptibility testing) | CO150 | 150 µg |
| **K. Sulphonamides and Similars** | | |
| SULPHONAMIDES* | SULFA | 240 µg |
| TRIMETHOPRIM | TRIM5 | 5 µg |
| TRIMETHOPRIM BSAC (U) | TP2.5 | 2.5 µg |
| TRIMETHOPRIM+SULFA | SxT25 | 1.25+23.75 µg |
| **L. Nitrofurans** | | |
| NITROFURANTOIN | NI300 | 300 µg |
| NITROFURANTOIN EUCAST | NI100 | 100 µg |
| NITROFURANTOIN BSAC (U) | NI200 | 200 µg |
| FURAZOLIDONE* | FURAZ | 50 µg |
| **M. Quinolone Derivatives** | | |
| NALIDIXAN | NAL30 | 30 µg |
| CIPROFLOXACIN 5 µg | CIPR5 | 5 µg |
| CIPROFLOXACIN 1 µg | CIPR1 | 1 µg |
| MOXIFLOXACIN | MOXIF | 5 µg |
| GATIFLOXACIN | GATIF | 5 µg |
| LEVOFLOXACIN | LEVOF | 5 µg |
| NORFLOXACIN | NORFX | 10 µg |
| NORFLOXACIN BSAC (U) | NOR.2 | 2 µg |
| OFLOXACIN | OFL.5 | 5 µg |
| **N. Others** | | |
| BACITRACIN (not for susceptibility testing) | BACIT | 40 µg |
| FOSFOMYCIN (+G6-P) | FO200 | 200 µg |
| FUCIDIN 10 µg EUCAST | FUC10 | 10 µg |
| METRONIDAZOLE 5 µg EUCAST | MTR.5 | 5 µg |
| METRONIDAZOLE 16 µg* | MTR16 | 16 µg |
| MUPIROCIN 10 µg* | MUPIR | 10 µg |
| MUPIROCIN 200 µg EUCAST | MP200 | 200 µg |
| NOVOBIOCIN 5 µg* | NOV05 | 5 µg |
| RETAPAMULIN | RETA2 | 2 µg |
| RIFAMPICIN | RIF.5 | 5 µg |
| **Special tests** | | |
| CLOXACILLIN (AmpC test) Diatabs** | CLOXA | - |
| PHENYLBORONIC ACID ( AmpC and KPC test) Diatabs** | BORON | - |
| DIPICOLINIC ACID (metallo-β- lactamases) Diatabs** | D.P.A | - |
| IMIPENEM+EDTA ** | IM10E | 10+750 µg |

**Note:**

* There are no potency recommendations from CLSI so far.
** Special potency tablets (discs) for detection of resistance mechanisms

# EUCAST-and CLSI potency NEO-SENSITABS™

## NEO-SENSITABS Range (Cartridges with Spring)



| Neo-Sensitabs | Identification Code Neo-Sensitabs | Diffusible Amount of Antimicrobial |
|---|---|---|
| AMPHOTERICIN B* | AMPHO | 10 µg |
| CICLOPIROX* | CICLO | 50 µg |
| CLOTRIMAZOLE* | CLOTR | 10 µg |
| ECONAZOLE* | ECONZ | 10 µg |
| FLUCONAZOLE | FLUCZ | 25 µg |
| 5-FLUOROCYTOSINE 1 µg* | FLU.1 | 1 µg |
| ISOCONAZOLE* | ISOCN | 10 µg |
| ITRACONAZOLE* | ITRAC | 10 µg |
| KETOCONAZOLE* | KETOC | 15 µg |
| MICONAZOLE* | MICON | 10 µg |
| NATAMYCIN* | NATAM | 50 µg |
| NYSTATIN* | NYSTA | 50 µg |
| TERBINAFINE* | TERBI | 30 µg |
| VORICONAZOLE | VOR.1 | 1 µg |
| CASPOFUNGIN | CASP5 | 5 µg |
| POSACONAZOLE | POSAC | 5 µg |

* There are no potency recommendations from CLSI so far.

ENTEROBACTERIACEAE

Zone diameter interpretative criteria and MIC breakpoints according to CLSI (formerly NCCLS) (chapter 1) when testing Enterobacteriaceae are listed in the table below. (1)

**Table 3.1-1 Interpretation for Enterobacteriaceae**

**Mueller-Hinton agar. Inoculum: McFarland 0.5. Incubation for 16-18 hours ambient air at 35°C ± 2 degrees.**

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| k) | Amikacin | 30 µg | AMI30 | ≥ 17 | 16-15 | ≤ 14 | ≤ 16 | ≥ 32 |
| | Amoxycillin+Clav. | 20+10 µg | AMC30 | ≥ 18 | 17-14 | ≤ 13 | ≤ 8/4 | ≥ 32/16 |
| e) | Ampicillin | 10 µg | AMP10 | ≥ 17 | 16-14 | ≤ 13 | ≤ 8 | ≥ 32 |
| | Ampicillin+Sulbactam | 10+10 µg | SAM20 | ≥ 15 | 14-12 | ≤ 11 | ≤ 8/4 | ≥ 32/16 |
| m) | Aztreonam | 30 µg | AZT30 | ≥ 21 | 20-18 | ≤ 17 | ≤ 4 | ≥ 16 |
| b) | ESBL screening | | | - | - | ≤ 27 | Screening ESBL | |
| d) | Cefazolin | 30 µg | CFZ30 | ≥ 23 | 22-20 | ≤ 19 | ≤ 2 | ≥ 8 |
| | Cefepime | 30 µg | FEP30 | ≥ 18 | 17-15 | ≤ 14 | ≤ 8 | ≥ 32 |
| | Cefepime+Clavulanate | 30+10 µg | FEP+C | Detection of ESBL | | | | |
| | Cefixime | 5 µg | CFM.5 | ≥ 19 | 18-16 | ≤ 15 | ≤ 1 | ≤ 1 |
| d) | Cefotaxime | 30 µg | CTX30 | ≥ 26 | 25-23 | ≤ 22 | ≥ 1 | ≥ 4 |
| b) | ESBL screening | | | - | - | ≤ 27 | Screening ESBL | |
| | Cefotaxime+Clav. | 30+10 µg | CTX+C | Detection of ESBL | | | | |
| | Cefoxitin | 30 µg | CFO30 | ≥ 18 | 17-15 | ≤ 14 | ≤ 8 | ≥ 32 |
| | Cefpodoxime | 10 µg | CPD10 | ≥ 21 | 20-18 | ≤ 17 | ≤ 2 | ≥ 8 |
| | ESBL screening | | | - | - | ≤ 17 | Screening ESBL | |
| | Ceftaroline | 30 µg | CPT30 | ≥ 23 | 22-20 | ≤ 19 | ≤ 0.5 | ≥ 2 |
| m) *) | Ceftazidime | 30 µg | CAZ30 | ≥ 21 | 20-18 | ≤ 17 | ≤ 4 | ≥ 16 |
| b) | ESBL screening | | | - | - | ≤ 22 | Screening ESBL | |
| | Ceftazidime+Clav. | 30+10 µg | CAZ+C | Detection of ESBL | | | | |
| | Ceftizoxime | 30 µg | ZOX30 | ≥ 25 | 24-22 | ≤ 21 | ≤ 1 | ≥ 4 |
| d) | Ceftriaxone | 30 µg | CTR30 | ≥ 23 | 22-20 | ≤ 19 | ≤ 1 | ≥ 4 |
| b) | ESBL screening | | | - | - | ≤ 25 | Screening ESBL | |
| | Cefuroxime (parenteral) | 30 µg | CXM30 | ≥ 18 | 17-15 | ≤ 14 | ≤ 8 | ≥ 32 |
| | Cefuroxime (oral) | 30 µg | CXM30 | ≥ 23 | 22-15 | ≤ 14 | ≤ 4 | ≥ 32 |
| d) | Cephalothin | 30 µg | CEP30 | ≥ 18 | 17-15 | ≤ 14 | ≤ 8 | ≥ 32 |
| | Chloramphenicol | 30 µg | CLR30 | ≥ 18 | 17-13 | ≤ 12 | ≤ 8 | ≥ 32 |
| | Ciprofloxacin | 5 µg | CIPR5 | ≥ 21 | 20-16 | ≤ 15 | ≤ 1 | ≥ 4 |
| c) | *Salmonella* spp. | | | ≥ 31 | 30-21 | ≤ 20 | ≤ 0.06 | ≥ 1 |
| | Ciprofloxacin | 1 µg | CIPR1 | | | | | |
| c) | *Salmonella* spp. | | | ≥ 26 | - | < 26 | ≤ 0.06 | ≥ 0.12 |
| h) | Cloxacillin | | CLOXA | Detection of plasmid mediated AmpC beta-lactamases | | | | |
| | Colistin | 10 µg | Co. 10 | | | | | |
| | 2+18 hour pre-diffusion | | | ≥ 15 | - | <15 | ≤ 2 | >4 |
| i) | Doxycycline | 30 µg | DOX30 | ≥ 14 | 13-11 | ≤ 10 | ≤ 4 | ≥ 16 |
| | Doripenem | 10 µg | DOR10 | ≥ 23 | 22-20 | ≤ 19 | ≤ 0.25 | ≥ 1 |
| j) | Ertapenem | 10 µg | ETP10 | ≥ 23 | 21-19 | ≤ 18 | ≤ 0.5 | ≥ 1 |

# EUCAST-and CLSI potency NEO-SENSITABS™

## Interpretation Zones and MIC Breakpoints according to CLSI

Enterobacteriaceae

| | | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|---|
| | | *Carbapenemase screen* | | | - | - | ≤ 22 | ≤ 0.5 | - (possible carbapenemase) |
| | | Fosfomycin (inj) | 200 µg | FO200 | ≥ 22 | 21-18 | < 18 | ≤ 16 | > 32 |
| a) | | Fosfomycin (U) | 200 µg | FO200 | ≥ 16 | 15-13 | ≤ 12 | ≤ 64 | ≥ 256 |
| | | Gatifloxacin | 5 µg | GATIF | ≥ 18 | 17-15 | ≤ 14 | ≤ 2 | ≥ 8 |
| k) | | Gentamicin | 10 µg | GEN10 | ≥ 15 | 14-13 | ≤ 12 | ≤ 4 | ≥ 8 |
| g) | j) | Imipenem | 10 µg | IMI10 | ≥ 23 | 22-20 | ≤ 19 | ≤ 1 | ≥ 4 |
| | | *Carbapenease screen* | | | - | - | <23 | ≤ 1 | - (possible carbapenemase) |
| g) | | Imipenem+EDTA | 10+750 µg | IM10E | Detection of metallo-ß-lactamases | | | | |
| | | Kanamycin | 30 µg | KAN30 | ≥ 18 | 17-14 | ≤ 13 | ≤ 6 | ≥ 25 |
| | | Levofloxacin | 5 µg | LEVOF | ≥ 17 | 16-14 | ≤ 13 | ≤ 2 | ≥ 8 |
| | | *Salmonella* spp. | | | ≥ 29 | 28-19 | ≤ 18 | ≤ 0.125 | ≥ 2 |
| a) | | Mecillinam (U) | 10 µg | MEC10 | ≥ 15 | 14-12 | ≤ 11 | ≤ 8 | ≥ 32 |
| j) | | Meropenem | 10 µg | MRP10 | ≥ 23 | 22-20 | ≤ 19 | ≤ 1 | ≥ 4 |
| | | *Carbapenemase screen* | | | - | - | <25 | ≤ 0.5 | - (possible carbapenemase) |
| i) | | Minocycline | 30 µg | MIN30 | ≥ 16 | 15-13 | ≤ 12 | ≤ 4 | ≥ 16 |
| | | Moxifloxacin | 5 µg | MOXIF | ≥ 19 | 18-16 | ≤ 15 | ≤ 2 | ≥ 8 |
| a) | | Nalidixan (U) | 30 µg | NAL30 | ≥ 19 | 18-14 | ≤ 13 | ≤ 16 | ≥ 32 |
| c) | | Screening quinolones | | | - | - | < 15 | - | Reduced suscept. to quinolones |
| | | Netilmicin | 30 µg | NET30 | ≥ 15 | 14-13 | ≤ 12 | ≤ 12 | ≥ 32 |
| a) | f) | Nitrofurantoin (U) | 300 µg | NI300 | ≥ 17 | 16-15 | ≤ 14 | ≤ 32 | ≥ 128 |
| a) | | Norfloxacin (U) | 10 µg | NORFX | ≥ 17 | 16-13 | ≤ 12 | ≤ 4 | ≥ 16 |
| | | Ofloxacin | 5 µg | OFL.5 | ≥ 16 | 15-13 | ≤ 12 | ≤ 2 | ≥ 8 |
| | | *Salmonella* spp. | | | | | | ≤ 0.12 | ≥ 2 |
| | | Piperacillin | 100 µg | PIPRA | ≥ 21 | 20-18 | ≤ 17 | ≤ 16 | ≥ 128 |
| | | Piperacillin+Tazobactam | 100+10µg | PI+TZ | ≥ 21 | 20-18 | ≤ 17 | ≤ 16/4 | ≥ 128/4 |
| | | Streptomycin | 10 µg | STR10 | ≥ 15 | 14-12 | ≤ 11 | - | - |
| a) | | Sulphonamides (U) | 240 µg | SULFA | ≥ 17 | 16-13 | ≤ 12 | ≤ 256 | ≥ 512 |
| l) | | Temocillin | 30 µg | TEMOC | ≥ 18 | 17-15 | ≤ 14 | ≤ 16 | ≥ 32 |
| i) | | Tetracyclines | 30 µg | TET30 | ≥ 15 | 14-12 | ≤ 11 | ≤ 4 | ≥ 16 |
| | | Ticarcillin | 75 µg | TIC75 | ≥ 20 | 19-15 | ≤ 14 | ≤ 16 | ≥ 128 |
| | | Ticarcillin+Clavulanate | 75+10 µg | TIM85 | ≥ 20 | 19-15 | ≤ 14 | ≤ 16/2 | ≥ 128/2 |
| n) | | Tigecycline | 15 µg | TIG15 | ≥ 19 | 18-15 | ≤ 14 | ≤ 2 | ≥ 8 |
| k) | | Tobramycin | 10 µg | TOB10 | ≥ 15 | 14-13 | ≤ 12 | ≤ 4 | ≥ 8 |
| a) | | Trimethoprim (U) | 5 µg | TRIM5 | ≥ 16 | 15-11 | ≤ 10 | ≤ 4 | ≥ 16 |
| | | Trimethoprim+Sulfa | 1.25+23.75 µg | SxT25 | ≥ 16 | 15-11 | ≤ 10 | ≤ 2/38 | ≥ 8/152 |
| | | Boronic acid | | BORON | Detection of AmpC and KPC Beta.lactamases | | | | |
| | | Dipicolinic acid | | D.P.A | Detection of metallo-ß-lactamases | | | | |

a) (U) For urinary tract infections only.

b) Strains of Klebsiella, *E. coli,* Salmonella that **produce ESBL**, may be clinically resistant to therapy with penicillins, cephalosporins or aztreonam, despite apparent in vitro susceptibility, see "Detection of resistance mechanisms using Neo-Sensitabs™ and DiaTabs™".
Strains showing zones ≤ 27 mm with Aztreonam and/or Cefotaxime, ≤ 17 mm with Cefpodoxime, ≤ 22 mm with Ceftazidime and/or ≤ 25 mm with Ceftriaxone should be suspected of producing ESBL.
For confirmatory tests, use Ceftazidime ± Clavulanate, Cefotaxime ± Clavulanate, Cefepime ± Clavulanate and/or Cefpodoxime ± Clavulanate.
An increase in zone diameter of ≥ 5 mm for the combination Cefpodoxime+Clavulanate, Cefotaxime+Clavulanate, Ceftazidime+Clavulanate or Cefepime+Clavulanate compared to Cefpodoxime/Cefotaxime/ Ceftazidime/Cefepime alone is confirmatory of the presence of an ESBL.
If resistant to cefotaxime, ceftazidime and ceftriaxone, negative for ESBL and susceptible for cefepime, report as found.
Using the new CLSI breakpoints for cephalosporins (9, 10) a substantial number of E. coli and P. mirabilis containing ESBLs would be reported as susceptible to CAZ, FEP and AZT.

For K. oxytoca and C. koseri showing **R** to Cefuroxime, Ceftriaxone and Piperacillin/Tazobactam, but susceptible to ceftazidime, are not ESBL producers (false positives may be obtained using 3rd generation Cephalosporins). Test for ESBL production with Ceftazidime and Clavulanate.

c) Strains resistant to nalidixic acid show a decreased susceptibility to quinolones (MIC CIPRO ≥ 0.125 µg/ml). Nalidixic makes a good screening for detection of decreased fluoroquinolone susceptibility in ***Salmonella* spp.** (4).
According to Hakanen et al. (2) and Parry et al. (7) *Salmonella enterica* isolates from Southeast Asia may show a new quinolone resistance pattern: NAL susceptible and CIPRO reduced susceptibility (MIC ≥ 0.12 µg/ml), therefore test both Nalidixan and Ciprofloxacin 1 µg Neo-Sensitabs.
If resistant to Ciprofloxacin, report as **R** to all fluoroquinolones.

d) For Enterobacteriaceae isolated from the **CSF test cefotaxime** (or ceftriaxone) instead of cephalothin (or cefazolin).

e) *Klebsiella* and *Enterobacter* spp. should always be reported as **R** to ampicillin.

f) *Klebsiella*, *Enterobacter* and *Proteus* spp. should always be reported as **R** to nitrofurantoin.

g) Isolates with MIC > 0.5 µg/ml against Imipenem and highly resistant to ceftazidime (KPC and GES enzymes) should be suspected of possessing carbapenemases. For detecting carbapenemases Ambler classes A and D. Synergy between BORON and carbapenems indicates KPC.
For detecting metallo-beta-lactamases test, Dipicolinic acid with Meropenem and Ertapenem and Imipenem+EDTA against Imipenem Neo-Sensitabs or use the KPC+MBL Confirm ID kit. Further information is giving in User's Guide "Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs™" (www.rosco.dk).

h) Enterobacteriaceae suspicious of possessing plasmid mediated Amp C beta lactamases (Cefoxitin R, Ceftazidime R, Cefepime S and Carbapenems S) should be tested for synergy between Boronic acid and Ceftazidime+Clavulanate and Cefotaxime+Clavulanate and/or between Cefotaxime/Ceftazidime and Cloxacillin 500 µg (distance 5-10 mm from edge to edge) by using combined Neo-Sensitabs disc tests (AmpC Confirm ID kit). See further details in "Detection of resistance mechanisms using Neo-Sensitabs™ and DiaTabs™" (www.rosco.dk).

i) Sader et al. recommends smaller inhibition zones for tetracyclines. (3)

j) Results with one carbapenem cannot be extrapolated to the other. If a strain is resistant to Imipenem or Meropenem, report as resistant to ertapenem.
Screen values for possible carbapenemases (metallo-beta-lactamases) are ≤ 23 mm for Ertapenem, Meropenem and Imipenem. (6)

k) - If I/R to Tobramycin and susceptible to Gentamicin, report Amikacin as **I** (Intermediate).

- If **I** to Gentamicin and susceptible to other aminoglycosides, report as Gentamicin **R**.

- If **I** to Tobramycin, Gentamicin **R** and Amikacin **S**, report as Tobramycin **R**.

- If Gentamicin **R** and Tobramycin **R**, report as resistant to netilmicin.

l) Rodriguez-Villalobos et al found that Temocillin Neo-Sensitabs showed better discrimination between susceptible and resistant and lower number of discrepancies than paper disks (BD). (5)

m) New CLSI proposed breakpoints for Cephalosporins, Aztreonam and Enterobacteriaceae (Teleconference, August 2008).

n) Tigecycline 15 µg disc has poor resolution (1-2 mm) between disc modal zones for adjacent MICs. (8)

**References:**

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2) Hakanen et al J. Clin. Microbiol., **43**, 5775-8, 2005.
3) Sader H.S. et al: Reevaluation of CLSI Disk diffusion breakpoints for Tetracyclines for testing Enterobacteriaceae. J. Clin. Microbiol., **45**, 1640-1643, 2007.
4) Aznar E. et al: Detection of decreased susceptibility to fluoroquinolones in Salmonella spp. by 5 different methods including real-time PCR. Int. J. Antimicr. Agents, **30**, 67-71, 2007**.**
5) Rodriguez-Villalobos H. et al: Comparison of 4 commercial methods for antimicrobial susceptibility testing of Temocillin. ECCMID, abstract R2411, Barcelona, april 2008.
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PSEUDOMONAS AERUGINOSA

Zone diameter interpretative criteria and MIC breakpoints according to CLSI (formerly NCCLS) (1) when testing *P. aeruginosa*, *Acinetobacter* spp., *S. maltophilia*, *B. cepacia*, are listed in the tables below.

**Table 3.2-1 Interpretation for *Pseudomonas aeruginosa***

**Mueller-Hinton Agar. Inoculum McFarland 0.5. Incubation at 35 °C ± 2 degrees ambient air for 16-18 hours.**

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| a) | Amikacin | 30 µg | AMI30 | ≥ 17 | 16-15 | ≤ 14 | ≤ 8 | ≥ 32 |
| | Aztreonam | 30 µg | AZT30 | ≥ 22 | 21-16 | ≤ 15 | ≤ 8 | ≥ 32 |
| | Cefepime | 30 µg | FEP30 | ≥ 18 | 17-15 | ≤ 14 | ≤ 8 | ≥ 32 |
| d) | Cefepime+Clavulanate | FEP+C | 30+10 µg | detection of ESBL | | | | |
| | Ceftazidime | 30 µg | CAZ30 | ≥ 18 | 17-15 | ≤ 14 | ≤ 8 | ≥ 32 |
| d) | Ceftazidime+Clavulanate | CAZ+C | 30+10µg | detection of ESBL | | | | |
| | Ciprofloxacin | 5 µg | CIPR5 | ≥ 21 | 20-16 | ≤ 15 | ≤ 1 | ≥ 4 |
| c) | Colistin | 10 µg | Co.10 | | | | | |
| c) | 2+18 hours' prediffusion method | | | ≥ 15 | 14-11 | ≤ 10 | ≤ 2 | ≥ 8 |
| f) | Doripenem | 10 µg | DOR10 | ≥ 19 | 18-17 | ≤ 16 | ≤ 2 | ≥ 8 |
| | Fosfomycin | 200 µg | FO200 | ≥ 22 | 21-19 | ≤ 18 | ≤ 16 | ≥ 32 |
| | Gatifloxacin | 5 µg | GATIF | ≥ 18 | 17-15 | ≤ 14 | ≤ 2 | ≥ 8 |
| a) | Gentamicin | 10 µg | GEN10 | ≥ 15 | 14-13 | ≤ 12 | ≤ 4 | ≥ 8 |
| e,f) | Imipenem | 10 µg | IMI10 | ≥ 19 | 18-16 | ≤ 15 | ≤ 2 | ≥ 8 |
| b) | Imipenem+EDTA | 10+750µg | IM10E | detection of metallo-ß-lactamases | | | | |
| | Levofloxacin | 5 µg | LEVOF | ≥ 17 | 16-14 | ≤ 13 | ≤ 2 | ≥ 8 |
| e,f) | Meropenem | 10 µg | MRP10 | ≥ 19 | 18-16 | ≤ 15 | ≤ 2 | ≥ 8 |
| | Minocycline | 30 µg | MIN30 | ≥ 19 | 18-15 | ≤ 14 | ≤ 4 | ≥ 16 |
| | Netilmicin | 30 µg | NET30 | ≥ 15 | 14-13 | ≤ 12 | ≤ 4 | ≥ 8 |
| | Ofloxacin (U) | 5 µg | OFL.5 | ≥ 16 | 15-13 | ≤ 12 | ≤ 2 | ≥ 8 |
| | Piperacillin | 100 µg | PIPRA | ≥ 21 | 20-15 | ≤ 14 | ≤ 16 | ≥ 128 |
| | Piperacillin+Tazobactam | 100+10µg | PI+TZ | ≥ 21 | 20-15 | ≤ 14 | ≤ 16/4 | ≥ 128/4 |
| | Tetracyclines | 30 µg | TET30 | ≥ 19 | 18-15 | ≤ 14 | ≤ 4 | ≥ 16 |
| | Ticarcillin | 75 µg | TIC75 | ≥ 20 | 19-15 | ≤ 14 | ≤ 16 | ≥ 128 |
| | Ticarcillin+Clavulanate | 75+10 µg | TIM85 | ≥ 24 | 23-17 | ≤ 16 | ≤ 16/2 | ≥ 128/2 |
| a) | Tobramycin | 10 µg | TOB10 | ≥ 15 | 14-13 | ≤ 12 | ≤ 2 | ≥ 8 |
| | Dipicolinic acid | | D.P.A | Detection of metallo-ß-beta lacta-mases | | | | |

- *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis requires incubation up to 24 hours.

Clinical isolates of *P. aeruginosa* heterogeneously resistant to carbapenems have been isolated (1). Subcolonies appearing within the zone of inhibition of Imipenem/Meropenem show higher MIC values. Automated systems and conventional agar dilution MICs using the standard $10^4$ CFU per spot inoculum may miss carbapenem-resistant mutants.
Detection of *P. aeruginosa* resistant to Colistin in University Hospital in Barcelona (3).

Unacceptable levels of error in beta-lactam susceptibility were detected using 4 automated methods (2). The authors suggest that clinical laboratories using automated systems should consider accurate alternative methods (agar diffusion methods) for routine use.

Cabot et al (4) conclude that isolates non-susceptible to Ceftazidime or Piperacillin-Tazobactam were found to hyperproduce AmpC,MexAB-OprM, while particularly MexX-OprM overexpression was found among Cefepime-non-susceptible isolates.
Overexpression of MexXY-OprM is known to confer low-level resistance to aminoglycosides.
All Imipenem resistant isolates showed inactivating mutations in oprD.

**References:**

1)Pournaras S. et al: Characterization of clinical isolates of P. aeruginosa heterogeneously resistant to carbapenems. J. Med. Microbiol, **56**, 66-70, 2007.
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Antimicr. Agents Chemother. **55**, 1906-1911, 2011.

ACINETOBACTER spp.

**Table 3.2-1**

**Interpretation for *Acinetobacter* spp.**

**Mueller-Hinton Agar. Inoculum McFarland 0.5. Incubation at 35 °C ± 2 degrees ambient air for 20-24 hours.**

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| a) | Amikacin | 30 µg | AMI30 | ≥ 17 | 16-15 | ≤ 14 | ≤ 16 | ≥ 32 |
| | Ampicillin +Sulbactam | 10+10 µg | SAM20 | ≥ 15 | 14-12 | ≤ 11 | ≤ 8/4 | ≥ 32/16 |
| | Aztreonam | 30 µg | AZT30 | ≥ 22 | 21-16 | ≤ 15 | ≤ 8 | ≥ 32 |
| | Cefepime | 30 µg | FEP30 | ≥ 18 | 17-15 | ≤ 14 | ≤ 8 | ≥ 32 |
| d) | Cefepime +Clavunalate | 30+10 µg | FEP+C | detection of ESBL | | | | |
| | Ceftazidime | 30 µg | CAZ30 | ≥ 18 | 17-15 | ≤ 14 | ≤ 8 | ≥ 32 |
| d) | Ceftazidime +Clavulanate | 30+10µg | CAZ+C | detection of ESBL | | | | |
| | Cefotaxime | 30 µg | CTX30 | ≥ 23 | 22-15 | ≤ 14 | ≤ 8 | ≥ 64 |
| | Ceftriaxone | 30 µg | CTR30 | ≥ 21 | 17-14 | ≤ 13 | ≤ 8 | ≥ 64 |
| | Chloramphenicol | CLR30 | 30 µg | ≥ 18 | 17-13 | ≤ 12 | ≤ 8 | ≥ 32 |
| | Ciprofloxacin | 5 µg | CIPR5 | ≥ 21 | 20-16 | ≤ 15 | ≤ 1 | ≥ 8 |
| c) | Colistin (7) | 10 µg | Co.10 | | | | | |
| c) | 2+18 hours' prediffusion | | | ≥ 15 | 14-11 | ≤ 10 | ≤ 2 | ≥ 8 |
| | Doripenem | 10 µg | DOR10 | ≥ 18 | 17-15 | ≤ 14 | ≤ 1 | ≥ 4 |
| | Doxycycline | 30 µg | DOX30 | ≥ 14 | 13-11 | ≤ 10 | ≤ 4 | ≥ 16 |
| | Gatifloxacin | 5 µg | GATIF | ≥ 18 | 17-15 | ≤ 14 | ≤ 2 | ≥ 8 |
| a) | Gentamicin | 10 µg | GEN10 | ≥ 15 | 14-13 | ≤ 12 | ≤ 4 | ≥ 8 |
| *) | Imipenem | 10 µg | IMI10 | ≥ 16 | 15-14 | ≤ 13 | ≤ 4 | ≥ 16 |
| b) | Imipenem +EDTA | 10+750 µg | IM10E | detection of metallo-ß-lactamases | | | | |
| | Levofloxacin | 5 µg | LEVOF | ≥ 17 | 16-14 | ≤ 13 | ≤ 2 | ≥ 8 |
| *) | Meropenem | 10 µg | MRP10 | ≥ 16 | 15-14 | ≤ 13 | ≤ 4 | ≥ 16 |
| | Minocycline | 30 µg | MIN30 | ≥ 16 | 15-13 | ≤ 12 | ≤ 4 | ≥ 16 |
| | Piperacillin | 100 µg | PIPRA | ≥ 21 | 20-18 | ≤ 17 | ≤ 16 | ≥ 128 |
| | Piperacillin +Tazobactam | 100+10 µg | PI+TZ | ≥ 21 | 20-18 | ≤ 17 | ≤ 16/4 | ≥ 128/4 |
| | Rifampicin | 5 µg | RIF.5 | ≥ 16 | 15-12 | ≤ 11 | ≤ 4 | ≥ 16 |
| | Tetracyclines | 30 µg | TET30 | ≥ 15 | 14-12 | ≤ 11 | ≤ 4 | ≥ 16 |
| | Ticarcillin | 75 µg | TIC75 | ≥ 20 | 19-15 | ≤ 14 | ≤ 16 | ≥ 128 |
| | Ticarcillin +Clavulanate | 75+10 µg | TIM85 | ≥ 20 | 19-15 | ≤ 14 | ≤ 16/2 | ≥ 128/2 |
| **) | Tigecycline | 15 µg | TIG15 | ≥ 22 | 21-20 | ≤ 19 | ≤ 1 | >2 |
| a) | Tobramycin | 10 µg | TOB10 | ≥ 15 | 14-13 | ≤ 12 | ≤ 4 | ≥ 8 |
| | Trimethoprim (U) | 5 µg | TRIM5 | ≥ 16 | 15-11 | ≤ 10 | | |
| | Trimethoprim +Sulfa | 1.25+23.75 µg | SxT25 | ≥ 16 | 15-11 | ≤ 10 | ≤ 2/38 | ≥ 8/152 |
| | Dipicolinic acid | | D.P.A | Detect. of | metallo - beta | lactama-ses | | |

Multiresistent *Acinetobacter baumannii*, resistant to all beta-lactams including imipenem/meropenem, should be suspected of producing carbapenemases; either Class D enzymes (oxacillinases OXA-23, OXA-24, OXA-58) or Class A enzymes (metallo-ß-lactamases).

There are differences in activity between tigecycline and minocycline, consequently, they should be tested separately (11).

Clinical isolates of Colistin hetero-resistant E.cloacae and A. baumannii were not detected by Vitek-2 (9). Colistin-dependent A. baumannii were not detected by broth microdilution. The strain grows around a 10 µg colistin disk.

*Screen values for possible carbapenemases (OXA and MBL) are <21mm for Ertapenem and <22mm for Meropenem and Imipenem.

**Tentative breakpoints. Oxoid MHA gives average 3.5 mm smaller zones than BD-MHA and should not be used for testing tigecycline (12).

## BURKHOLDERIA CEPACIA AND S. MALTOPHILIA

**Table 3.2-2**

**Interpretation for *Burkholderia cepacia***

**Mueller-Hinton Agar. Inoculum McFarland 0.5. Incubation at 33-35°C ambient air for 20-24 hours.**

| NEO-SENSITABS CODE | POTENCY | | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|
| Ceftazidime | 30 µg | CAZ30 | ≥ 21 | 20-18 | ≤ 17 | ≤ 8 | ≥ 32 |
| c) Colistin | 10 µg | Co.10 | | | | | |
| c) 2+18 hours' prediffusion | | | ≥ 15 | 14-11 | ≤ 10 | ≤ 2 | ≥ 4 |
| Doripenem | 10 µg | DOR10 | ≥ 20 | 19-16 | ≤ 15 | ≤ 4 | ≥ 16 |
| Meropenem | 10 µg | MRP10 | ≥ 20 | 19-16 | ≤ 15 | ≤ 4 | ≥ 16 |
| Temocillin | 30 µg | TEMOC | ≥ 18 | 17-15 | ≤ 14 | ≤ 16 | ≥ 32 |
| Tigecycline | 15 µg | TIG15 | ≥ 20 | 19-17 | ≤ 16 | ≤ 2 | ≥ 8 |
| Trimethoprim+ Sulfa | 1.25+23.75µg | SxT25 | ≥ 16 | 15-11 | ≤ 10 | ≤ 2/38 | ≥ 8/152 |

**Table 3.2-3**

**Interpretation for *Stenotrophomonas maltophilia***

**Mueller-Hinton Agar. Inoculum McFarland 0.5. Incubation at 33-35°C ambient air for 20-24 hours.**

| NEO-SENSITABS CODE | POTENCY | | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|
| Fosfomycin (+ G6P) | 200 µg | FO200 | ≥ 20 | - | - | ≤ 32 | - |
| Levofloxacin | 5 µg | LEVOF | ≥ 17 | 16-14 | ≤ 13 | ≤ 2 | ≥ 8 |
| Minocycline | 30 µg | MIN30 | ≥ 19 | 18-15 | ≤ 14 | ≤ 4 | ≥ 16 |
| Moxifloxacin | 5 µg | MOXIF | ≥ 18 | 17-15 | ≤ 14 | ≤ 1 | ≥ 4 |
| Tigecycline | 15 µg | TIG15 | ≥ 20 | 19-17 | ≤ 16 | ≤ 2 | ≥ 8 |
| Trimethoprim +Sulfa | 1.25+23.75µg | SxT25 | ≥ 16 | 15-11 | ≤ 10 | ≤ 2/38 | ≥ 8/152 |

a)*S. maltophilia* is intrinsic resistant towards aminoglycosides (report R).
For *S. maltophilia* and *B. cepacia* incubation at 30 °C for 24 hours might be more appropriate for detecting beta lactam resistance (2, 5, 8).
For Pseudomonas if **I/R** to Tobramycin and susceptible to Gentamicin, report Amikacin as resistant.
Resistance to Trimethoprim + Sulfamethazole is increasing in Brazil (27%). (13)

b)For detection of carbapenemases (including metallo-beta-lactamases), see "Detection of resistance mechanisms using Neo-Sensitabs™ and DiaTabs™".

c)For accurate detection of colistin resistance in multidrug resistant strains, use the 2+18 hours' prediffusion method. This technique permits a good separation between susceptible and resistant strains: Place Colistin 10 µg Neo-Sensitabs on a non-inoculated Mueller-Hinton plate and incubate for 2 hours at room temperature (≤25°C). Thereafter eliminate the tablet by knocking the plate against a table and leave the plate for 18 hours at room temperature of ≤25°C (next day). Inoculate the plate with the strain to be tested. Incubate for 18-24 hours at 33-35 °C and read the inhibition zones (see Ch. 1.5).
Colistin 10 µg disk testing without prediffusion can be used as screening test for high level resistance with *P. aeruginosa* (MIC ≥ 128 µg/ml corresponds to no zone of inhibition).

d) For detection of strains producing ESBLs. Further information in User's Guide "Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs™".

e)For Acinetobacter/Pseudomonas: results for one carbapenem (Imipenem, Meropenem) cannot be extrapolated to others.

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STAPHYLOCOCCI

Zone diameter interpretative criteria and MIC breakpoints according to CLSI (formerly NCCLS) (1) when testing staphylococci are listed in the table below.

**Table 3.3-1 Interpretation for *Staphylococcus* spp.**

**Mueller-Hinton agar. Inoculum: McFarland 0.5. Incubation for 16-18 hours ambient air at 35 °C ± 2 degrees, however, incubate for Oxacillin and Vancomycin for 24 hours at 33-35 °C.**

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| d) | Amikacin | 30 µg | AMI30 | ≥ 17 | 16-15 | ≤ 14 | ≤ 16 | ≥ 32 |
| | Amoxycillin+Clav. | 20+10 µg | AMC30 | Test Cefoxitin | | | | |
| | Ampicillin | 10 µg | AMP10 | Test Penicillin | | | | |
| i) | Azithromycin | 15 µg | AZI15 | ≥ 18 | 17-14 | ≤ 13 | ≤ 2 | ≥ 8 |
| | Cefoxitin (10,11,13,16) | **10 µg** | CFO10 | | | | | |
| | *S. aureus/S. lugdunensis* | | | ≥ 17 | - | ≤ 16 | - | mecA pos. |
| | Coagulase neg. staph. | | | ≥ 21 | - | ≤ 20 | - | mecA pos. |
| a) b) | Cefoxitin | **30 µg** | CFO30 | | | | | |
| | *S. aureus/S. lugdunensis* | | | ≥ 22 | - | ≤ 21 | ≤ 4 | mecA pos. |
| | Coagulase neg. staph. | | | ≥ 25 | - | ≤ 24 | ≤ 0.25 (oxa) | ≥ 0.5 |
| b) | Cefepime | 30 µg | FEP30 | Test Cefoxitin | | | ≤ 8 | ≥ 32 |
| b) | Cefotaxime | 30 µg | CTX30 | Test Cefoxitin | | | ≤ 8 | ≥ 64 |
| | Ceftaroline | 30 µg | CPT30 | ≥ 24 | 23-21 | ≤ 20 | ≤ 1 | ≥ 4 |
| b) | Ceftazidime | 30 µg | CAZ30 | Test Cefoxitin | | | ≤ 8 | ≥ 32 |
| a) | Ceftizoxime | 30 µg | ZOX30 | | | | | |
| | *S. aureus* | | | ≥ 20 | - | ≤ 15 | Oxa S | mecA pos. (Oxa R) |
| b) | Ceftriaxone | 30 µg | CTR30 | Test Cefoxitin | | | ≤ 8 | ≥ 64 |
| b) | Cefuroxime parenteralt | 30 µg | CXM30 | Test Cefoxitin | | | ≤ 8 | ≥ 32 |
| b) | Cefuroxime oral | 30 µg | CXM30 | Test Cefoxitin | | | ≤ 4 | ≥ 32 |
| b) | Cephalothin | 30 µg | CEP30 | Test Cefoxitin | | | ≤ 8 | ≥ 32 |
| | Chloramphenicol | 30 µg | CLR30 | ≥ 18 | 17-13 | ≤ 12 | ≤ 8 | ≥ 32 |
| | Ciprofloxacin | 5 µg | CIPR5 | ≥ 21 | 20-16 | ≤ 15 | ≤ 1 | ≥ 4 |
| | Clarithromycin | 15 µg | CLA15 | ≥ 18 | 17-14 | ≤ 13 | ≤ 2 | ≥ 8 |
| i) | Clindamycin | 2 µg | CLI.2 | ≥ 21 | 20-15 | ≤ 14 | ≤ 0.5 | ≥ 4 |
| h) | Daptomycin | 30 µg | DAPCa | | | | | |
| | 2+18 hours' prediffusion | | | ≥ 22 | - | < 20 | ≤ 1 | ≥ 2 |
| | Doripenem | 10 µg | DOR10 | ≥ 30 | - | - | ≤ 0.5 | - |
| | Doxycycline | 30 µg | DOX30 | ≥ 16 | 15-13 | ≤ 12 | ≤ 4 | ≥ 16 |
| i) | Erythromycin | 15 µg | ERY15 | ≥ 23 | 22-14 | ≤ 13 | ≤ 0.5 | ≥ 8 |
| | Fucidin | 10 µg | FUC10 | ≥ 21 | - | ≤ 20 | ≤ 1 | > 1 |
| | Fucidin | 100 µg | FUCID | ≥ 28 | 27-24 | ≤ 23 | ≤ 1 | ≥ 4 |
| c) | Gatifloxacin | 5 µg | GATIF | ≥ 23 | 22-20 | ≤ 19 | ≤ 0.5 | ≥ 2 |
| d) | Gentamicin | 10 µg | GEN10 | ≥ 15 | 14-13 | ≤ 12 | ≤ 4 | ≥ 8 |
| b) | Imipenem | 10 µg | IMI10 | Test Cefoxitin | | | ≤ 4 | ≥16 |
| e) d) | Kanamycin | 30 µg | KAN30 | ≥ 18 | 17-14 | ≤ 13 | ≤ 6 | ≥ 25 |
| c) | Levofloxacin | 5 µg | LEVOF | ≥ 19 | 18-16 | ≤ 15 | ≤ 1 | ≥ 4 |
| j) | Linezolid | 30 µg | LINEZ | ≥ 21 | - | - | ≤ 4 | - |
| b) | Meropenem | 10 µg | MRP10 | Test Cefoxitin | | | ≤ 4 | ≥16 |
| | Minocycline | 30 µg | MIN30 | ≥ 19 | 18-15 | ≤ 14 | ≤ 4 | ≥ 16 |
| c) | Moxifloxacin | 5 µg | MOXIF | ≥ 24 | 23-21 | ≤ 20 | ≤ 0.5 | ≥ 2 |
| | Mupirocin | 10 µg | MUPIR | ≥ 16 | 15-10 | - | ≤ 4 | 128-LLR |
| | Staphylococci | | | - | - | no zone | | >256-HLR |
| d) f) | Netilmicin | 30 µg | NET30 | ≥ 15 | 14-13 | ≤ 12 | ≤ 12 | ≥ 32 |
| | Nitrofurantoin (U) | 300 µg | NI300 | ≥ 17 | 16-15 | ≤ 14 | ≤ 32 | ≥ 128 |
| | Novobiocin | 5 µg | NOVO5 | ≥ 14 | - | ≤ 13 | - | - |

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| c) | Norfloxacin (U) | 10 µg | NORFX | ≥ 17 | 16-13 | ≤ 12 | ≤ 4 | ≥ 16 |
| c) | Ofloxacin | 5 µg | OFL.5 | ≥ 18 | 17-15 | ≤ 14 | ≤ 1 | ≥ 4 |
| a) | Oxacillin | 1 µg | OXA.1 | | | | | |
| | *S. aureus/ S. lugdunensis* | | | ≥ 13 | 12-11 | ≤ 10 | ≤ 2 | ≥ 4 |
| | Coagulase neg. staph. | | | ≥ 18 | - | ≤ 17 | ≤ 0.25 | ≥ 0.5 |
| a) | Penicillin | 10 U | PEN10 | ≥ 29 | - | ≤ 28 | ≤ 0.12 | beta lactamase |
| | Quinupristin-Dalfoprist. | 15 µg | SYN15 | ≥ 19 | 18-16 | ≤ 15 | ≤ 1 | ≥ 4 |
| | Retapamulin | 2 µg | RETA2 | ≥ 20 | 19-17 | ≤ 16 | ≤ 0.5 | ≥ 2 |
| | Rifampicin | 5 µg | RIF.5 | ≥ 20 | 19-17 | ≤ 16 | ≤ 1 | ≥ 4 |
| | Sulphonamides (U) | 240 µg | SULFA | ≥ 17 | 16-13 | ≤ 12 | ≤ 100 | ≥ 350 |
| | Teicoplanin | 30 µg | TPN30 | | | | | |
| g) | 2+18 hours' prediffusion (BHI blood) | | | ≥ 18 | - | <18 | ≤ 4 | GISA/TRCNS |
| | 2+18 hours' prediffusion, MH plain | | | ≥ 22 | - | < 20 | ≤ 4 | GISA |
| | Telithromycin | 15 µg | TEL15 | ≥ 22 | 21-19 | ≤ 18 | ≤ 1 | ≥ 4 |
| | Tetracyclines | 30 µg | TET30 | ≥ 19 | 18-15 | ≤ 14 | ≤ 4 | ≥ 16 |
| | Tigecycline | 15 µg | TIG15 | ≥ 19 | - | - | ≤ 0.5 | - |
| d) | Tobramycin | 10 µg | TOB10 | ≥ 15 | 14-13 | ≤ 12 | ≤ 4 | ≥ 8 |
| | Trimethoprim (U) | 5 µg | TRIM5 | ≥ 16 | 15-11 | ≤ 10 | ≤ 4 | ≥ 16 |
| | Trimethoprim+Sulfa | 1.25+23.75 µg | SxT25 | ≥ 16 | 15-11 | ≤ 10 | ≤ 2/38 | ≥ 8/152 |
| | Vancomycin | 30 µg | VAN30 | - | - | No zone | - | >32 VRSA |
| g) | 2+18 hours' prediffusion (BHI blood) | | | ≥ 22 | - | < 20 | ≤ 2 | VISA/GISA |
| | 2+18 hours' prediffusion, MH plain | | | ≥ 21 | - | ≤ 20 | ≤ 2 | VISA/GISA |

a) Penicillin zone edge test: Robert Skov presented data showing, that the Penicillin zone edge test was more sensitive than the Nitrocefin test, using Penicillin 10 unit disc. Sharp zone edges indicate beta-lactamase production, while fussy zone edges indicate no beta-lactamase production.
Staphylococci and beta-lactams: test only Penicillin, Cefoxitin (Ceftizoxime) and Oxacillin.
Isolates that test as mecA negative (Cefoxitin S), but which show Oxacillin total resistance (no zone), should be reported as resistant.
Isolates that test mecA positive and are Cefoxitin S and Oxacillin S, should be reported as susceptible to Oxacillin (PBP not functioning properly).
If both Cefoxitin and Oxacillin are tested against S. aureus or S. lugdunensis and either result is resistant, the organism is reported as Oxacillin resistant.
mecA-independent beta-lactam resistance (17) (Cefoxitin S, Oxacillin R, Ceftobiprole R) is likely to become more important as Ceftobiprole/Ceftaroline are developed and used clinically. Therefore test both Cefoxitin and Oxacillin. If Oxacillin resistant, report the isolate as resistant regardless of the result given by Cefoxitin.

b) Cefoxitin and *S. aureus* icubation 18-24 h at 35 °C. For coagulase neg. staph. incubate for 24 h. Results may be reported after 18 h, if resistant (10, 13).
Cefoxitin (Oxacillin) resistant staphylococci should be considered resistant to all available beta-lactam antibiotics and combinations with beta-lactamase inhibitors.

c) There is cross resistance between quinolones against staphylococci.

d) Staphylococci resistant to Gentamicin should be reported as resistant to Netilmicin, Tobramycin and Amikacin (APH (2'') + AAC (6')).
Tobramycin sensitive strains will currently be susceptible to Gentamicin and Netilmicin.
Strains resistant to Tobramycin, are reported as resistant to Kanamycin and Amikacin.

e) Interpretation valid for Amikacin and Isepamicin. Isolates resistant to Kanamycin should be reported as resistant to Amikacin (no matter zone size around Amikacin)

f) Netilmicin may give false sensitive results with coagulase negative staphylococci. Use Gentamicin. Isolates sensitive to Gentamicin will currently be susceptible to Netilmicin.

g) The CLSI Staphylococci Working Group has agreed to remove the disk diffusion zone breakpoint for Staphylococci, because the current diffusion method does not distinguish between Vanco S and hVISA or VISA strain. VRSA can be detected with the current diffusion method (without prediffusion) because they produce no zone of inhibition around Vancomycin 30 µg Neo-Sensitabs™.

For detection of hVISA/GISA/VISA strains use the 2+18 hours' prediffusion method with Teicoplanin 30 µg and Vancomycin 30 µg (see "Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs™"). TRCNS = Teicoplanin resistant coagulase negative staphylococci (ex. *S. haemolyticus* (8)). Fitzgibbon et al: (12) showed that 5 µg Teicoplanin on BHI agar and MCF 0.5 inoculum, gave the best results as screening method for h-GISA strains.

h) For detection of Daptomycin resistance, use the 2+18 hours' prediffusion method (see "Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs™").

i) Results with Erythromycin are also valid for Clarithromycin and Azithromycin.
Macrolide-resistant isolates may have inducible clindamycin resistance that can be detected by the **D-zone test**, using Erythromycin 15 µg and Clindamycin 2 µg placed approximately 15 mm apart (edge to edge).

j) For reading the inhibition zone, use transmitted light and measure the inner edge of the zone of inhibition.
Staphylococci showing **R** to Linezolid, Chloramphenicol, Clindamycin and Quinu/Dalfopristin may possess cfr methyltransferases (15).

Vancomycin susceptibility among S.aureus (14)

| Vancomycin | Name | MIC µg/ml |
|---|---|---|
| Susceptible | VSSA | ≤ 2 µg/ml |
| Heteroresistant* | hVISA/hGISA | 1-2 µg/ml |
| Intermediate | VISA/GISA | 4-8 µg/ml |
| Resistant | VRSA | ≥ 16 µg/ml |

*Consist of sub-populations (≤ $10^{-6}$) that may grow in media containing >2 µg/ml Vancomycin. For detecting hVISA/hGISA and VISA/GISA use the 2+18 hour pre-diffusion method with Vancomycin 30 µg/ml and Teicoplanin 30 µg/ml Neo-Sensitabs.
Infections involving hVISA are a problem, since these strains are reported by laboratories to be susceptible to vancomycin on the basis of current recommended MIC breakpoints. Therefore, the true prevalence of hVISA is unknown.

Stegger et al (18) found that isolates containing mecA(LGA251), now called mecC, are phenotypically resistant to beta-lactams, but have not been recognized as classical MRSA with conventional PCRs for mecA, owing to the different nucleotide composition.The mecC producing isolates were resistant to Cefoxitin, indicating that the diffusion method with Cefoxitin is reliable for any type of S. aureus.

Kotsakis et al (19) describe the emergence of a mecA negative S. lugdunensis with a modified PBP 1A/1B that expresses resistance to all beta-lactams.The strain was resistant to Oxacillin and Cefoxitin, but was not detected by the mecA test.

Lindquist et al (20) demonstrates the presence of pseudo-SCC elements resembling SCCmec type II among Multiresistant-MSSA. All 54 isolates were PCR positive for the nuc gene and negative by the mecA gene. Methods testing the nuc gene (GeneOhm MRSA) gave false resistant results.

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**SCREENING TESTS FOR STAPHYLOCOCCI**

**Inoculum: Mc Farland 0.5 and incubation at 35°C for 18-24 hours**

| Test | Organism group | Neo-S potency | Test method | Medium | Interpretation |
|---|---|---|---|---|---|
| Beta-lactamase | Staphylo-cocci | Penicillin 10 units | Disk diffusion | MH | R:≤28 mm |
| Oxacillin res. mecA | S.aureus<br>S.lugdu-nensis<br><br>Coagulase neg. staph. | Cefoxitin 30 µg and Oxacillin 1 µg | Disk diffusion | MH | S:≥22 mm R:≤21 mm (cefoxitin)<br>S≥:13 mm R:≤10 mm (oxacillin)<br><br>S:≥25 mm R:≤24 mm (cefoxitin)<br>S:≥18 mm R:≤17 mm (oxacillin) |
| Vancomycin/Teicoplanin reduced susceptibility | S.aureus<br>Coagulase neg. staph. | Vancomy-cin 30 µg<br>Teicopla-nin 30 µg | Pre-diffusion 2+18 hours | BHI+5% blood or MH plain | Vanco: R:<22 mm (hVISA/VISA/GISA)<br><br>Teico: R:<20 mm (hVISA/VISA/GISA) TRCNS R<18 mm (BHI blood) |
| Daptomycin reduced susceptibility | S.aureus | Daptomy-cin 30 µg | Pre-diffusion 2+18 hours | MH | S: ≥22 mm R:<20 mm |
| Inducible Clindamycin resistance (D-zone) | Staphylo-cocci | Erythro-mycin 15 µg<br>Clinda-mycin 2 µg | Disk diffusion | MH | Flattering of the disk adjacent to the erythromycin disk =inducible clindamycin resistance. Report clindamycin **R** |
| Aminoglycosides | Staphylo-cocci | Kanamy-cin 30 µg<br>Gentamy-cin 10 µg<br>Tobramy-cin 10 µg | Disk diffusion | MH | Kana 30: S: ≥18 R: ≤ 13<br>Genta 10: S: ≥15 R: ≤ 12<br>Tobra 10: S: ≤ 15 R: ≤ 12<br><br>Results with kanamycin are valid for amikacin and isepamicin.<br>Strains resistant to genta are reported as resistant to tobra, netilmicin and amikacin<br>(APH ($2^n$) + AAC ($6^1$).<br>Isolates susceptible to gentamicin will currently be susceptible to netilmicin.<br>Strains resistant to tobramycin are reported as resistant to kanamycin and amikacin. |

ENTEROCOCCI

Zone diameter interpretative criteria and MIC breakpoints according to CLSI (formerly NCCLS) (1) when testing enterococci are listed in the table below.

Ligu et al (9) isolated 23 vanA genotype VREF and found that 12 of them (52%) had vanB phenotype (Teicoplanin MICs 2-12 µg/ml).

**Table 3.4-1 Interpretation for *Enterococcus* spp.**

**Mueller-Hinton agar. Inoculum: McFarland 0.5. Incubation for 18-24 hours ambient air at 35 °C ± 2 degrees.**

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| e) | Ampicillin | 10 µg | AMP10 | ≥ 17 | - | ≤ 16 | ≤ 8 | ≥ 16 |
| | Chloramphenicol | 30 µg | CLR30 | ≥ 18 | 17-13 | ≤ 12 | ≤ 8 | ≥ 32 |
| | Ciprofloxacin (U) | 5 µg | CIPR5 | ≥ 21 | 20-16 | ≤ 15 | ≤ 1 | ≥ 4 |
| g) | Daptomycin | 30 µg | DAPCa | ≥ 12 | - | - | ≤ 4 | - |
| | 2+18 hours' prediffusion | | | ≥ 12 | - | - | ≤ 4 | - |
| | Doxycycline | 30 µg | DOX30 | ≥ 16 | 15-13 | ≤ 12 | ≤ 4 | ≥ 16 |
| f) | Erythromycin | 15 µg | ERY15 | ≥ 23 | 22-14 | ≤ 13 | ≤ 0.5 | ≥ 8 |
| | Fosfomycin (U) | 200 µg | FO200 | ≥ 16 | 15-13 | ≤ 12 | ≤ 64 | ≥ 256 |
| | Gatifloxacin (U) | 5 µg | GATIF | ≥ 18 | 17-15 | ≤ 14 | ≤ 2 | ≥ 8 |
| h) | Imipenem | 10 µg | IMI10 | ≥ 16 | 15-14 | ≤ 13 | ≤ 4 | ≥ 16 |
| | Levofloxacin (U) | 5 µg | LEVOF | ≥ 17 | 16-14 | ≤ 13 | ≤ 2 | ≥ 8 |
| | Linezolid | 30 µg | LINEZ | ≥ 23 | 22-21 | ≤ 20 | ≤ 2 | ≥ 8 |
| | Minocycline | 30 µg | MIN30 | ≥ 19 | 18-15 | ≤ 14 | ≤ 4 | ≥ 16 |
| | Nitrofurantoin (U) | 300 µg | NI300 | ≥ 17 | 16-15 | ≤ 14 | ≤ 32 | ≥ 128 |
| | Norfloxacin (U) | 10 µg | NORFX | ≥ 17 | 16-13 | ≤ 12 | ≤ 4 | ≥ 16 |
| | Penicillin | 10 U | PEN10 | ≥ 15 | - | ≤ 14 | ≤ 8 | ≥ 16 |
| a) | Quinupristin/Dalfopristin | 15 µg | SYN15 | ≥ 19 | 18-16 | ≤ 15 | ≤ 1 | ≥ 4 |
| | Rifampicin | 5 µg | RIF.5 | ≥ 20 | 19-17 | ≤ 16 | ≤ 1 | ≥ 4 |
| c) | Teicoplanin | 30 µg | TPN30 | ≥ 14 | 13-11 | ≤ 10 | ≤ 8 | ≥ 32 |
| | 2+18 hour pre-diffusion | | | ≥ 16 | - | <16 | ≤ 8 | TRE |
| | Tetracyclines | 30 µg | TET30 | ≥ 19 | 18-15 | ≤ 14 | ≤ 4 | ≥ 16 |
| | Tigecycline | 15 µg | TIG15 | ≥ 19 | - | - | ≤ 0.25 | - |
| c) | Vancomycin | 30 µg | VAN30 | >16 | 15-14 | < 14 | ≤ 4 | ≥ 32 |
| | (2+18 hours' prediffusion) | | | ≥16 | 15-12 | < 12 | ≤ 4 | VRE |
| b) d) | Gentamicin | 250 µg | GN250 | - | - | < 14 | - | HLR |
| b) | Streptomycin | 500 µg | ST500 | - | - | < 14 | - | HLR |

a) *E. faecalis* is intrinsically resistant to Quinupristin/Dalfopristin.

b) For detection of High Level Aminoglycoside Resistance (HLR) use high level Neo-Sensitabs (Gentamicin 250 µg and Streptomycin 500 µg).

c) With Vancomycin and Teicoplanin plates should be incubated for full 24 hours and examined carefully. Vancomycin resistant strains show a hazy zone edge while sensitive strains show a sharp zone edge.

d) Gentamicin HLR in enterococci indicates resistance to all aminoglycosides, except Streptomycin. *E. faecium* shows intrinsic resistance towards Kanamycin, Tobramycin and Netilmicin due to the production of the enzyme AAC (6').

e) Results with Ampicillin and *E. faecalis* are valid for Amoxicillin and Piperacillin. Reduced susceptibility to Ampicillin is common in *E. faecium*. If resistant to Ampicillin, report as resistant to ureidopenicillins. Martin et al. (10) report Ampicillin S)/Imipenem R and Ampicillin R/Imipenem S in 5% of clinical isolates.

f) Results with Erythromycin are valid also for Azithromycin and Clarithromycin.

g) For detection of Daptomycin resistance, use the 2+18 hours' prediffusion method (valid only for *E. faecalis* susceptible to Vancomycin).

h) Ampicillin susceptible but Penicillin (MIC ≥ 16 µg/ml) and Imipenem (MIC ≥ 8 µg/ml) resistant strains of *E. faecalis* were widely disseminated in a Greek hospital (31.4% with this phenotype)(7,8). Ampicillin and Imipenem should be tested separately. Guardabassi et al. (11) found the same pattern in Denmark.

   For more information on glycopeptide resistance see: "Detection of resistance mechanisms using Neo-Sensitabs™ and DiaTabs™".

Gagnon et al (12) found during routine screening for VRE 6 Enterococcus faecium isolates positive for vanA by PCR, but susceptible to Vancomycin and Teicoplanin by phenotypic testing.
The 6 isolates were missing the vanR and vanS genes that are responsible for the activation of resistance genes. False resistance results with PCR testing.

Sarti et al (13) describe the isolation of 8 beta-lactamase-producing multiresistant E. faecium isolates from 2010. The beta-lactamase activity was demonstrated at a high inoculum and in the presence of Methicillin after overnight incubation.

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PNEUMOCOCCI

Zone diameter interpretative criteria and MIC breakpoints according to CLSI (formerly NCCLS) (1) when testing S*treptococcus pneumoniae* are listed in the table below.

The current tablet diffusion procedure described for Neo-Sensitabs should be followed, but no more than 9 Neo-Sensitabs should be placed on a large (150 mm) plate or 4 Neo-Sensitabs on a 90-100 mm plate. The recommended medium is Mueller-Hinton agar supplemented with 5% defibrinated sheep blood.

Soriano et al (9) found S. pneumoniae isolates with penicillin/cefotaxime, MICs of 16 µg/ml, in strains from Romania.

**Table 3.5-1 Interpretation for *Streptococcus pneumoniae***

**Mueller-Hinton + 5 % blood. Inoculum: McFarland 0.5. Incubation at 35 °C ± 2 degrees in 5-7 % $CO_2$ for 20-24 hours.**

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| | Azithromycin | 15 µg | AZI15 | ≥ 18 | 17-14 | ≤ 13 | ≤ 0.5 | ≥ 2 |
| | Ceftaroline | 30 µg | CPT30 | ≥ 26 | - | - | ≤ 0.5 | - |
| a) | Ceftizoxime (Cefotaxime, Ceftriaxone) | 30 µg | ZOX30 | ≥ 26 | - | ≤ 25 | ≤ 0.5 (3rd gen. cepha.) | - |
| | Chloramphenicol | 30 µg | CLR30 | ≥ 21 | - | ≤ 20 | ≤ 4 | ≥ 8 |
| | Clarithromycin | 15 µg | CLA15 | ≥ 21 | 20-17 | ≤ 16 | ≤ 0.25 | ≥ 1 |
| f) | Clindamycin | 2 µg | CLI.2 | ≥ 19 | 18-16 | ≤ 15 | ≤ 0.25 | ≥ 1 |
| | Doripenem | 10 µg | DOR10 | - | - | - | ≤ 1 | - |
| | Doxycycline | 30 µg | DOX30 | ≥ 28 | 27-25 | ≤ 24 | ≤ 0.25 | ≥ 1 |
| f) | Erythromycin | 15 µg | ERY15 | ≥ 21 | 20-16 | ≤ 15 | ≤ 0.25 | ≥ 1 |
| e) | Gatifloxacin | 5 µg | GATIF | ≥ 21 | 20-18 | ≤ 17 | ≤ 1 | ≥ 4 |
| | Imipenem | 10 µg | IMI10 | ≥ 28 | 27-25 | ≤ 24 | ≤ 0.12 | ≥ 1 |
| e) | Levofloxacin | 5 µg | LEVOF | ≥ 17 | 16-14 | ≤ 13 | ≤ 2 | ≥ 8 |
| | Linezolid | 30 µg | LINEZ | ≥ 21 | - | - | ≤ 2 | - |
| | Meropenem | 10 µg | MER10 | ≥ 26 | 25-23 | ≤ 22 | ≤ 0.25 | ≥ 1 |
| e) | Moxifloxacin | 5 µg | MOXIF | ≥ 18 | 17-15 | ≤ 14 | ≤ 1 | ≥ 4 |
| e) | Norfloxacin | 10 µg | LEVOF | | | < 12 | Reduced Susceptibility to quinolones | >8 |
| | Ofloxacin | 5 µg | OFL.5 | ≥ 16 | 15-13 | ≤ 12 | ≤ 2 | ≥ 8 |
| b) | Oxacillin (penicillin) | 1 µg | OXA.1 | ≥ 20 | ≤ 19 | ≤ 19 | ≤ 0.06 (pen) | ≥ 0.12 (I/R) (pen) |
| | Quinupristin/Dalfopristin | 15 µg | SYN15 | ≥ 19 | 18-16 | ≤ 15 | ≤ 1 | ≥ 4 |
| | Rifampicin | 5 µg | RIF.05 | ≥ 19 | 18-17 | ≤ 16 | ≤ 1 | ≥ 4 |
| | Teicoplanin | 30 µg | TPN30 | ≥ 16 | - | - | ≤ 1 | - |
| | Telithromycin | 15 µg | TEL15 | ≥ 19 | 18-16 | ≤ 15 | ≤ 1 | ≥ 4 |
| g) | Tetracyclines | 30 µg | TET30 | ≥ 24 | 23-21 | ≤ 20 | ≤ 1 | ≥ 4 |
| | Tigecycline | 15 µg | TIG15 | ≥ 19 | - | - | ≤ 0.25 | - |
| | Trimethoprim +Sulfa | 1.25+23.75 µg | SxT25 | ≥ 19 | 18-16 | ≤ 15 | ≤ 0.5/9.5 | ≥ 4/76 |
| | Vancomycin | 30 µg | VAN30 | ≥ 17 | - | - | ≤ 1 | - |
| c) | **non-meningeal criteria** | | | | | | | |
| | Amoxycillin+Clav. | 20+10 µg | AM+CL | ≥ 23 | 22-20 | ≤ 19 | ≤ 2/1 | ≥ 8/4 |
| | Ampicillin (valid for Amoxycillin and Penicillin G) | 10 µg | AMP10 | ≥ 20 | 19-17 | ≤ 16 | ≤ 2 | ≥ 8 |
| | Oxacillin (valid for Penicillin-Voral) | 1 µg | | ≥ 20 | 19-10 | no zone | ≤ 0.06 | ≥ 2 |
| | Cefepime | 30 µg | FEP30 | ≥ 24 | 23-22 | ≤ 21 | ≤ 1 | ≥ 4 |

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| d) | Cefotaxime | 30 µg | CTX30 | ≥ 24 | 23-22 | ≤ 21 | ≤ 1 | ≥ 4 |
| | Cefpodoxime | 10 µg | CPD10 | ≥ 23 | 22-19 | ≤ 18 | ≤ 0.5 | ≥ 2 |
| | Ceftriaxone | 30 µg | CTR30 | ≥ 24 | 23-22 | ≤ 21 | ≤ 1 | ≥ 4 |
| | Cefuroxime (oral) | 30 µg | CXM30 | ≥ 24 | 23-22 | ≤ 21 | ≤ 1 | ≥ 4 |

a) Cefotaxime and Ceftriaxone must not be tested against pneumococci (CSF) by the diffusion method. A surrogate test: Ceftizoxime is used instead. Ceftizoxime detects reduced sensitivity to 3rd and 4th generation cephalosporins.

b) Oxacillin 1 µg is used for screening susceptibility towards Penicillin is reported as S or I/R to Penicillin (not Oxacillin).
Non-meningeal pneumococcal isolates with Penicillin MIC ≤ 2 µg/ml (Ampicillin 10 µg zone ≥ 20 mm) can be considered susceptible to Amoxycillin, Amoxycillin+ Clavulanate, Cefepime, Cefotaxime, Ceftriaxone and Ertapenem (1).
Non-meningeal pneumococci with Penicillin MIC ≤ 0.06 µg/ml (Oxacillin zone ≥ 20 mm) can also be considered susceptible to Ampicillin (parenteral), Ampicillin+ Sulbactam, Imipenem and Meropenem.
Penicillin and Cefotaxime/Ceftriaxone or Meropenem MIC's should be determined for isolates with Oxacillin zones ≤ 19mm (1).

c) For use in acute otitis media, acute sinusitis and comunity acquired pneumonia (non-meningitis).

d) Interpretation criteria for Cefotaxime requires doses appropriate for serious pneumococcal infections; e.g. at least 1 g (adults) or 50 mg/kg (children) every 8 hours or more frequently.

- Penicillin-resistant strains from the CSF should be considered resistant to Ampicillin, Amoxycillin, Amoxycillin+Clavulanate, and first and second generation cephalosporins.

- Emergence in USA of pneumococci with very high level resistance to Penicillin (MIC ≥ 8 µg/ml), associated with high Amoxicillin MIC's (≥ 8 µg/ml) and high Cefotaxime MIC's.

- Chiu et al (7) observed an increasing Ceftriaxone resistance in *S. pneumoniae* from Taiwan.

e) Currently used CLSI MIC breakpoints for fluoroquinolones define many pneumococci isolates as susceptible even though they harbour QRDR mutations (8). Microbiological resistance breakpoints are as follows: Levofloxacin (MIC > 1 µg/ml ~ zone ≤ 20 mm), Moxifloxacin (MIC > 0.12 µg/ml ~ zone ≤ 25 mm), Gatifloxacin (MIC > 0.25 µg/ml ~ zone ≤ 24 mm) and Norfloxacin (MIC>8 µg/ml ~ zone <12mm). If resistant to Moxifloxacin, reports as resistant to all fluoroquinolones.
Isolates susceptible to Levofloxacin are susceptible to Moxifloxacin, but not vice versa.

- CLSI recommends Penicillin, Cefotaxime (or Ceftriaxone) and Meropenem to be tested by a reliable MIC method and reported routinely with **CSF isolates** of *S. pneumoniae*.

f) Macrolide-resistant isolates may have inducible Clindamycin resistance, which can be detected by the D-zone test (See 3.3.0 page 5).

g) According to Rosco's Regression line analysis the following interpretation corresponds to the recommended MIC's: S ≥ 24 mm, I: 23-21 mm, R ≤ 20 mm

**References:**


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Zone diameter interpretative criteria and MIC breakpoints according to CLSI (formerly NCCLS) (1) when testing *Streptococci* other than *S. pneumoniae* are listed in the table below

The current tablet diffusion procedure described for Neo-Sensitabs should be followed, but no more than 9 Neo-Sensitabs should be placed on a large (150 mm) plate or 4 Neo-Sensitabs on a 90-100 mm plate. The recommended medium is Mueller-Hinton agar supplemented with 5% defibrinated sheep blood.

Small colony-forming beta-haemolytic strains with group A, C, F or G antigens (S. anginosus, previously termed *S. milleri*) are considered part of the viridans group. The viridans group also includes: *S. mitis*, *S. oralis*, *S. sanguinis*, *S. salivarius*, *S. intermedius*, *S. constellatus*, *S. mutans* and *S. bovis*.

**Table 3.6-1**

**Interpretation for Streptococci (except *S. pneumoniae*)**

**Mueller-Hinton + 5 % blood. Inoculum: McFarland 0.5. Incubation at 35 °C ± 2 degrees in 5-7 % $CO_2$ for 20-24 hours.**

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| a) | Ampicillin | 10 µg | AMP10 | | | | | |
| | Beta-haemolytic | | | ≥ 24 | - | - | ≤ 0.25 | - |
| | Viridans | | | ≥ 24 | 23-17 | ≤ 16 | ≤ 0.25 | ≥ 8 |
| c) e) | Azithromycin | 15 µg | AZI15 | ≥ 18 | 17-14 | ≤ 13 | ≤ 0.5 | ≥ 2 |
| | Cefepime | 30 µg | FEP30 | | | | | |
| | Beta-haemolytic | | | ≥ 24 | - | - | ≤ 0.5 | - |
| | Viridans | | | ≥ 24 | 23-22 | ≤ 21 | ≤ 1 | ≥ 4 |
| | Cefotaxime | 30 µg | CTX30 | | | | | |
| | Beta-haemolytic | | | ≥ 24 | - | - | ≤ 0.5 | - |
| | Viridans | | | ≥ 28 | 27-24 | ≤ 23 | ≤ 1 | ≥ 4 |
| | Ceftaroline | 30 µg | CPT30 | | | | | |
| | *S. pyogenes* | | | ≥ 26 | - | - | ≤ 0.5 | - |
| | *S. agalactiae* | | | ≥ 26 | - | - | ≤ 0.5 | - |
| | Ceftibuten (screen penicillin group B) | 30 µg | CTB30 | ≥ 20 | - | <20 | - | ≥ 0.25 |
| | Ceftizoxime (screen penicillin group B) | 30 µg | ZOX30 | - | - | <30 | - | ≥ 0.25 |
| | Ceftriaxone | 30 µg | CTR30 | | | | | |
| | Beta-haemolytic | | | ≥ 24 | - | - | ≤ 0.5 | - |
| | Viridans | | | ≥ 27 | 26-25 | ≤ 24 | ≤ 1 | ≥ 4 |
| | Chloramphenicol | 30 µg | CLR30 | ≥ 21 | 20-18 | ≤ 17 | ≤ 4 | ≥ 16 |
| c) e) | Clarithromycin | 15 µg | CLA15 | ≥ 21 | 20-17 | ≤ 16 | ≤ 0.25 | ≥ 1 |
| c) | Clindamycin | 2 µg | CLI.2 | ≥ 19 | 18-16 | ≤ 15 | ≤ 0.25 | ≥ 1 |
| | Daptomycin | 30 µg | DAPCa | | | | | |
| | 2+18 h prediffusion | | | ≥ 22 | - | - | ≤ 1 | - |
| | Doripenem | 10 µg | DOR10 | | | | | |
| | Beta-haemolytic | | | ≥ 24 | - | - | ≤ 0.12 | - |
| | Doxycycline (viridans) | 30 µg | DOX30 | ≥ 23 | 22-19 | ≤ 18 | ≤ 2 | ≥ 8 |
| c) | Erythromycin | 15 µg | ERY15 | ≥ 21 | 20-16 | ≤ 15 | ≤ 0.25 | ≥ 1 |
| | Gatifloxacin | 5 µg | GATIF | ≥ 21 | 20-18 | ≤ 17 | ≤ 1 | ≥ 4 |

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| | Imipenem | 10 µg | IMI10 | ≥ 26 | 25-23 | ≤ 22 | ≤ 0.25 | ≥ 1 |
| | Levofloxacin | 5 µg | LEVOF | ≥ 17 | 16-14 | ≤ 13 | ≤ 2 | ≥ 8 |
| | Linezolid | 30 µg | LINEZ | ≥ 21 | - | - | ≤ 2 | - |
| | Meropenem | 10 µg | MER10 | ≥ 26 | 25-23 | ≤ 22 | ≤ 0.5 | - |
| | Minocycline | 30 µg | MIN30 | ≥ 23 | 22-19 | ≤ 18 | ≤ 2 | ≥ 8 |
| | Moxifloxacin | 5 µg | MOXIF | ≥ 21 | 20-18 | ≤ 17 | ≤ 1 | ≥ 4 |
| | Ofloxacin | 5 µg | OFL.5 | ≥ 16 | 15-13 | ≤ 12 | ≤ 2 | ≥ 8 |
| b) | Oxacillin (penicillin screening) | 1 µg | OXA.1 | ≥ 14 | ≤ 13 | ≤ 13 | ≤ 012 (pen) | ≥ 0.25 (I/R) |
| a) | Penicillin | 10 U | PEN10 | | | | | |
| | Beta-haemolytic | | | ≥ 24 | - | - | ≤ 0.12 | - |
| | Viridans | | | ≥ 26 | 25-13 | ≤ 12 | ≤ 0.12 | ≥ 4 |
| | Quinupristin/Dalfopristin | 15 µg | SYN15 | ≥ 19 | 18-16 | ≤ 15 | ≤ 1 | ≥ 4 |
| | Retapamulin | 2 µg | RETA2 | ≥ 15 | - | - | ≤ 0.25 | - |
| | Rifampicin | 5 µg | RIF.5 | ≥ 20 | 19-17 | ≤ 16 | ≤ 1 | ≥ 4 |
| | Teicoplanin | 30 µg | TPN30 | ≥ 16 | - | - | ≤ 1 | - |
| | Telithromycin | 15 µg | TEL15 | ≥ 19 | 18-16 | ≤ 15 | ≤ 1 | ≥ 4 |
| | Tetracyclines | 30 µg | TET30 | ≥ 23 | 22-19 | ≤ 18 | ≤ 2 | ≥ 8 |
| | Tigecycline | 15 µg | TIG15 | ≥ 19 | - | - | ≤ 0.25 | - |
| | Trimethoprim | 5 µg | TRIM5 | ≥ 17 | 16-15 | ≤ 14 | ≤ 2 | > 2 |
| f) | Trimethoprim+Sulfa | 1.25+23.75 µg | SxT25 | ≥ 19 | 18-16 | ≤ 15 | ≤ 0.5/9.5 | ≥ 4/76 |
| | Vancomycin | 30 µg | VAN30 | ≥ 17 | - | - | ≤ 1 | - |

a) Viridans streptococci isolated from blood or CSF should be tested for Penicillin or Ampicillin susceptiblity using an MIC method.

b) Oxacillin 1 µg Neo-Sensitabs is useful for screening for Penicillin susceptibility in streptococci.

c) Macrolide resistant isolates may have inducible Clindamycin resistance, which can be detected by the D-zone test (See 3.3.0 page 5). Group B streptococci are susceptible to Ampicillin, Penicillin and Cefazolin, but may be resistant to Clindamycin and/or Erythromycin. In case of severe Penicillin allergy they should be tested and reported (D-zone test).
Bonfiglio et al (10) found S.agalactiae, Erythromycin S and Clindamycin R.

d) If resistant to Levofloxacin, report as resistant to all fluoroquinolones.

e) Result for Erythromycin is also valid for Clarithromycin and Azithromycin.

The first report of group B streptococci with confirmed reduced susceptibility to Penicillin (MIC 0.12-0.25 µg/ml) in blood isolates from Hong Kong (7).
Kimura et al (8,11) found that Ceftizoxime and Ceftibuten are the best surrogates for detecting Group B streptococci with reduced susceptibility to Penicillin, using the diffusion method.
Marvaud et al (9) reported the emergence of high level resistance to Gentamicin in S.pyogenes.

f) Please note that S. pyogenes (group A) and S. agalactiae (group B) are currently resistant to Trimethoprim+Sulfa.

Nagano et al (12) isolated multiple group B Streptococci with reduced Penicillin susceptibility, suspecting a possible nosocomial spread. They showed Penicillin MICs 0.25 to 1 µg/ml and were resistant to Ceftizoxime and Ceftibuten (good screening for detecting them in routine). In Canada the development of Penicillin non-susceptibility in group B streptococci has been described in vivo in adult patients.

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Zone diameter interpretative criteria and MIC breakpoints according to CLSI (formerly NCCLS) (1) when testing *Haemophilus* spp. are listed in the table below.

Note that too high inoculum may lead to false-resistant results with some beta-lactam antibiotics. Apply in general no more than 9 Neo-Sensitabs to the surface of a 140-150 mm plate and no more than 4 Neo-Sensitabs on a 90-100 mm plate.

The zone margin should be considered as the area showing no obvious growth visible with the unaided eye. Faint growth, or tiny colonies that may appear to fade from the more obvious zone, should be ignored in the measurement (1).

**Table 3.7-1 Interpretation for *Haemophilus influenzae/parainfluenzae***

**HTM-agar. Inoculum: McFarland 0.5. Incubation at 35-37 °C in 5-7 % $CO_2$ for 16-18 hours.**

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| | Amoxycillin+Clav | 2+1 µg | AMC.3 | ≥ 16 | - | < 16 | - | - |
| | Amoxycillin+Clav. | 20+10 µg | AMC30 | ≥ 20 | - | ≤ 19 | ≤ 4/2 | ≥ 8/4 |
| b) | Ampicillin | 2 µg | AMP.2 | ≥ 18 | 17-16 | < 16 | ≤ 1 | ≥ 4 |
| | Ampicillin | 10 µg | AMP10 | ≥ 22 | 21-19 | ≤ 18 | ≤ 1 | ≥ 4 |
| | Ampicillin+Sulbactam | 10+10 µg | SAM20 | ≥ 20 | - | ≤ 19 | ≤ 2/1 | ≥ 4/2 |
| c) | Azithromycin | 15 µg | AZI15 | ≥ 12 | - | - | ≤ 4 | - |
| | Aztreonam | 30 µg | AZT30 | ≥ 26 | - | - | ≤ 2 | - |
| | Cefaclor | 30 µg | CCL30 | ≥ 22 | 21-17 | ≤ 16 | ≤ 8 | ≥ 32 (amp R) |
| c) | Cefepime | 30 µg | FEP30 | ≥ 26 | - | - | ≤ 2 | - |
| c) | Cefixime | 5 µg | CFM30 | ≥ 21 | - | - | ≤ 1 | - |
| c) | Cefotaxime | 30 µg | | ≥ 26 | - | - | ≤ 2 | - |
| | Cefonicid | 30 µg | CFCID | ≥ 20 | 19-17 | ≤ 16 | ≤ 4 | ≥ 16 |
| c) | Cefpodoxime | 10 µg | CPD10 | ≥ 21 | - | - | ≤ 2 | - |
| | Ceftaroline | 30 µg | CPT30 | ≥ 30 | - | - | ≤ 0.5 | - |
| c) | Ceftazidime | 30 µg | CAZ30 | ≥ 26 | - | - | ≤ 2 | - |
| c) | Ceftizoxime | 30 µg | ZOX30 | ≥ 26 | - | - | ≤ 2 | - |
| | Ceftriaxone | 30 µg | CTR30 | ≥ 26 | - | - | ≤ 2 | - |
| | Cefuroxime | 30 µg | CXM30 | ≥ 20 | 19-17 | ≤ 16 | ≤ 4 | ≥ 16 |
| | Cephalothin | 30 µg | CEP30 | ≥ 22 | - | ≤ 21 | ≤ 8 | (amp R) |
| | Chloramphenicol | 30 µg | CLR30 | ≥ 29 | 28-26 | ≤ 25 | ≤ 2 | ≥ 8 |
| a) c) | Ciprofloxacin | 5 µg | CIPR5 | ≥ 21 | - | - | ≤ 1 | - |
| | Clarithromycin | 15 µg | CLA15 | ≥ 13 | 12-11 | ≤ 10 | ≤ 8 | ≥ 32 |
| | Doripenem | 10 µg | DOR10 | ≥ 16 | - | - | ≤ 1 | - |
| | Doxycycline | 30 µg | DOX30 | ≥ 27 | 26-24 | ≤ 23 | ≤ 2 | ≥ 8 |
| | Ertapenem | 10 µg | ETP10 | ≥ 19 | | | ≤ 0.5 | |
| a) c) | Gatifloxacin | 5 µg | GATIF | ≥ 18 | - | - | ≤ 1 | - |
| c) | Imipenem | 10 µg | IMI10 | ≥ 16 | - | - | ≤ 4 | - |
| a) | Levofloxacin | 5 µg | LEVOF | ≥ 17 | - | - | ≤ 2 | - |
| c) | Meropenem | 10 µg | MRP10 | ≥ 20 | - | - | ≤ 0.5 | - |
| a) | Moxifloxacin | 5 µg | MOXIF | ≥ 18 | - | - | ≤ 1 | - |
| a) | Nalidixan (screen quinolones) | 30 µg | NAL30 | - | - | < 19 | - | decreased suscept. to quinolones |
| a) c) | Ofloxacin | 5 µg | OFL.5 | ≥ 16 | - | - | ≤ 2 | - |
| | Penicillin | 10 U | PEN10 | ≥ 20 | 19-17 | ≤ 16 | ≤ 1 | ≥ 4 |
| | Piperacillin/Tazobactam | | | ≥ 21 | - | - | ≤ 1 | |
| | Rifampicin | 5 µg | RIF.5 | ≥ 20 | 19-17 | ≤ 16 | ≤ 1 | ≥ 4 |

| NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | Zone diameter in mm I | Zone diameter in mm R | Break-points MIC µg/ml S | Break-points MIC µg/ml R |
|---|---|---|---|---|---|---|---|
| Telithromycin | 15 µg | TEL15 | ≥ 15 | 14-12 | ≤ 11 | ≤ 4 | ≥ 16 |
| Tetracyclines | 30 µg | TET30 | ≥ 29 | 28-26 | ≤ 25 | ≤ 2 | ≥ 8 |
| Tigecycline (8) | 15 µg | TIG15 | ≥ 19 | - | - | ≤ 1 | - |
| Trimethoprim | 5 µg | TRIM5 | ≥ 23 | 22-20 | ≤ 19 | ≤ 1 | ≥ 4 |
| Trimethoprim+Sulfa | 1.25+23.75µg | SxT25 | ≥ 16 | 15-11 | ≤ 10 | ≤ 0.5/9.5 | ≥ 4/76 |

a) Strains resistant to nalidixic acid as well as strains showing zones < 30 mm with the quinolones should be suspected of having reduced susceptibility to quinolones. The current CLSI/NCCLS MIC breakpoints seem too high for detecting low level resistance.

b) Ampicillin 2 µg Neo-Sensitabs best detect the beta-lactamase-negative, Ampicillin-resistant (BLNAR) strains. BLNAR should be reported as resistant to Amoxycillin +Clav., Ampicillin+Sulbactam as well as to 1st and 2nd generation cephalosporins (Cefaclor, Cefuroxime).
Garcia-Cobos et al (10) in Spain (2007) found an increase to 12.8% of BLNAR strains.
Garcia-Cobos et al (11) suggests new Amoxicillin breakpoints in order to detect BLNAR isolates:
MIC ≤ 0.5 µg/ml (**S**, no resistance mechanisms), MIC 1-2 µg/ml (**I**, treatable) and MIC ≥ 4 µg/ml (**R**)
Ampicillin 2 µg will show zones of inhibition ≥18 mm (MIC ≤0.5 µg/ml). Cefaclor and Cephalexin (12) are also effective in detecting BLNAR strains.

The majority of *H. influenzae* that are resistant to Ampicillin and Amoxycillin are beta-lactamase producing strains (plasmidic TEM or ROB-1).
Beta-lactamase producing strains are easily detected
1) with a rapid beta-lactamase test e.g. Beta-lactamase (acido) Diagnostic Tablets (ROSCO Ref. 45521), using several colonies, because producers and non-producers may coexist in the sample,
2) using Amoxycillin+Clavulanate Neo-Sensitabs compared to Amoxycillin alone (same potency). Synergism (larger zone with Amoxycillin+Clavulanate) will be seen in the presence of a beta-lactamase.

ROB-1 resistance is widespread in Australia, including Cefaclor resistant strains (13).
PBP3 mutations that conferred reduced susceptibility to Amoxicillin and 3rd generation cephalosporins were found in clinical H. parainfluenzae isolates (15). Inhibitor-resistant TEM-34 was first detected in this pathogen.

Differentiation of strains showing beta-lactam resistance:

| ***H. influenzae*** | **AMP 2** | **AM+CL 20+10 µg** | **Beta-lactamase** | **Cefpodoxime+Clav.** | **Cefaclor** |
|---|---|---|---|---|---|
| Susceptible | Zone > 18 mm | Zone S | Neg. | No synergism | ≥ 23 mm |
| BLNAR | Zone < 18 mm | - | Neg. | No synergism | ≥ 22 mm |
| Beta-lactamase pos. TEM, ROB-1 | No zone | Zone > 20 mm (S) | Pos. | No synergism | ≥ 23 mm Variable (ROB-1) |
| AM+CL resistant BLPACR | No zone | Zone < 20 mm | Pos. | No synergism | zone < 20 mm |
| ESBL positive | No zone | S or R | Pos/neg. | Synergism | V |

BLPACR isolates were defined as AMC, MIC ≥ 4 µg/ml. Both BLNAR and BLPACR are susceptible to Ceftobiprole (14) and Ceftaroline.

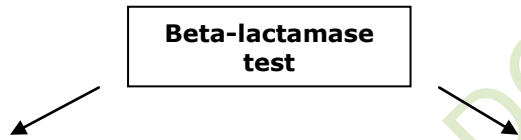
c) The CLSI has not yet defined other categories than "S" due to the current absence of resistant strains.

d) If Gentamicin susceptible, report susceptible to Amikacin, Tobramycin and Netilmicin.

e) *H. influenzae* tested against Pip/Tazo demonstrated uniformly high susceptibility (MIC ≤ 0.5 µg/ml) including BLNAR strains worldwide (9) and BLPACR.

Søndergaard et al (16) conclude that: Ampicillin 2 µg and Penicillin 1 Unit show an excellent performance in detecting BLNAR isolates. Discs containing Amox + Clav 2 + 1 µg could be used for the detection of N526K in beta-lactamase-producing isolates (BLPACR).
Algorithm: Penicillin 1 unit zones >= 12 mm are considered susceptible to penicillins/cephalosporins. Smaller zones are tested for beta-lactamase. Beta-lactamase positive showing zones < 23 mm with Cefaclor 30 µg are considered positive for mutational resistance and considered resistant to Cefuroxime.

Carcia-Cobos et al (17) have studied how to detect the different beta-lactam resistance mechanisms in H. influenzae. The algorithm could be:




| Negative | Positive |
|---|---|
| Penicillin 1U NEO-SENSITABS | Cefuroxime 30 µg NEO-SENSITABS |
| S ≥ 12 mm = susceptible | S ≥ 26 mm = BLPAR (MIC ≤ 1 µg/ml) |
| R ≤ 11 mm = BLNAR | R < 25 mm = BLPACR (MIC ≥ 2 µg/ml) |
| | or |
| | Amoxycillin+Clav 2+1 µg NEO-SENSITABS |
| | S ≥ 16 mm = BLPAR (MIC ≤ 2 µg/ml) |
| | R < 16 mm = BLPACR (MIC > 2 µg/ml) |

BLNAR **=** Beta-lactamase negative Ampicillin resistant
BLPAR = Beta-lactamase positive Ampicillin resistant
BLPACR = Beta-lactamase positive Amoxycillin + Clavulanate resistant

**References:**

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Zone diameter interpretative criteria and breakpoints when testing *Moraxella catarrhalis* are listed in the table below. The CLSI (formerly NCCLS) has now established MIC breakpoints or zone-diameter interpretative standards for *Moraxella catarrhalis*.

**Table 3.8-1 Interpretation for *Moraxella catarrhalis***

**Mueller-Hinton + 5 % blood+20 mg/l β-NAD. Inoculum: McFarland 0.5. Incubation for 16-18 hours at 35 °C ± 2 degrees. $CO_2$ may be used.**

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| | Amoxycillin+Clav. | 20+10 µg | AMC30 | ≥ 20 | - | ≤ 19 | ≤ 4/2 | ≥ 8/4 |
| a) | Ampicillin | 10 µg | AMP10 | ≥ 30 | 29-27 | ≤ 26 | ≤ 0.12 | penase |
| | Azithromycin | 15 µg | AZI15 | ≥ 18 | 17-13 | ≤ 12 | ≤ 2 | ≥ 8 |
| | Cefuroxime (oral) | 30 µg | CXM30 | ≥ 28 | 27-24 | ≤ 23 | ≤ 1 | ≥ 4 |
| | Chloramphenicol | 30 µg | CLR30 | ≥ 26 | 25-23 | ≤ 22 | ≤ 2 | ≥ 8 |
| | Ciprofloxacin | 5 µg | CIPR5 | ≥ 24 | - | ≤ 23 | ≤ 1 | - |
| | Clarithromycin | 15 µg | CLA15 | ≥ 18 | 17-13 | ≤ 12 | ≤ 2 | ≥ 8 |
| | Clindamycin | 2 µg | CLI.2 | ≥ 20 | 19-17 | ≤ 16 | ≤ 0.5 | ≥ 4 |
| | Doxycycline | 30 µg | DOX30 | ≥ 24 | 23-21 | ≤ 20 | ≤ 2 | ≥ 8 |
| | Erythromycin | 15 µg | ERY15 | ≥ 22 | 21-17 | ≤ 16 | ≤ 0.5 | ≥ 8 |
| | Gatifloxacin | 5 µg | GATIF | ≥ 30 | 29-27 | ≤ 26 | ≤ 0.25 | ≥ 0.5 |
| | Levofloxacin | 5 µg | LEVOF | ≥ 22 | - | ≤ 21 | ≤ 2 | - |
| | Moxifloxacin | 5 µg | MOXIF | ≥ 30 | 29-27 | ≤ 26 | ≤ 0.25 | ≥ 0.5 |
| | Nalidixan screen quinolones | 30 µg | NAL30 | - | - | < 18 | - | Reduced susceptibility to quinolones |
| | Ofloxacin | 5 µg | OFL.5 | ≥ 24 | - | ≤ 23 | ≤ 1 | - |
| a) | Penicillin | 10 U | PEN10 | ≥ 32 | 31-27 | ≤ 26 | ≤ 0.06 | penase |
| | Tetracyclines | 30 µg | TET30 | ≥ 24 | 23-21 | ≤ 20 | ≤ 2 | ≥ 8 |
| | Trimethoprim+Sulfa | 1.25+23.75µg | SxT25 | ≥ 16 | 15-11 | ≤ 10 | ≤ 0.5/9.5 | ≥ 2/38 |

a) Beta-lactamase-positive strains may be detected using Amoxycillin+Clavulanate Neo-Sensitabs and Ampicillin Neo-Sensitabs. Synergism (>8 mm larger zone with Amoxycillin+Clavulanate) is seen in the presence of beta-lactamases (BRO-1 or BRO-2). They should be reported as resistant to Penicillin, Ampicillin, Amoxycillin, Ticarcillin and Piperacillin.

b) Report resistant to Penicillin, Ampicillin and Amoxicillin (rare strains do not produce penicillinase).

**References:**


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Zone diameter interpretative criteria and MIC breakpoints when testing meningococci are listed in the table below. The CLSI (formerly NCCLS) has established MIC breakpoints for meningococci, and inhibition zone interpretative standards have now been approved by CLSI (1).

**Table 3.9-1 Interpretation for *Neisseria meningitidis***

**Mueller-Hinton agar + 5 % blood. McFarland 0.5. Incubation at 35 °C ± 2 degrees with 5-7 % $CO_2$ for 20 to 24 hours.**

| | | | | Zone diameter in mm | | | Break-points MIC µg/ml | |
|---|---|---|---|---|---|---|---|---|
| | NEO-SENSITABS | POTENCY | CODE | S | I | R | S | R |
| | Cefotaxime | 30 µg | CTX30 | ≥ 34 | - | - | ≤ 0.12 | - |
| | Ceftriaxone | 30 µg | CTR30 | ≥ 34 | - | - | ≤ 0.12 | - |
| | Chloramphenicol | 30 µg | CLR30 | ≥ 26 | 25-20 | ≤ 19 | ≤ 2 | ≥ 8 |
| | Meropenem | 10 µg | MRP10 | ≥ 30 | - | - | ≤ 0.25 | - |
| c) | Oxacillin (screen penicillin) | 1 µg | OXA.1 | ≥ 10 | - | - | ≤0.06 (pen) | MIC |
| e) | Mecillinam (Screen pen./amp.) | 10 µg | MEC10 | | | | | |
| | | | | ≥ 23 | - | ≤ 22 | ≤0.06 (pen) | - |
| | | | | ≥ 23 | - | ≤ 22 | ≤0.12 (amp) | - |
| a) | Azithromycin | 15 µg | AZI15 | ≥ 20 | - | - | ≤ 2 | - |
| a) | Ciprofloxacin | 5 µg | CIPR5 | ≥ 35 | 34-33 | ≤ 32 | ≤ 0.03 | ≥ 0.12 |
| | Ciprofloxacin | 1 µg | CIPR1 | ≥ 30 | 29-26 | < 26 | ≤ 0.03 | ≥ 0.12 |
| a) | Doxycycline | 30 µg | DOX30 | ≥ 26 | - | - | ≤ 2 | - |
| a) | Levofloxacin | 5 µg | LEVOF | ≥ 35 | 34-33 | ≤ 32 | ≤ 0.03 | ≥ 0.12 |
| a) | Minocycline | 30 µg | MIN30 | ≥ 26 | - | - | ≤ 2 | - |
| d) | Nalidixan (screen quinolones) | 30 µg | NAL30 | - | - | ≤ 25 | - | ≥ 8 |
| a) | Ofloxacin | 5 µg | OFL.5 | ≥ 35 | 34-33 | ≤ 32 | ≤ 0.03 | ≥ 0.12 |
| a) | Rifampicin | 5 µg | RIF.5 | ≥ 25 | 24-20 | ≤ 19 | ≤ 0.5 | ≥ 2 |
| a) b) | Trimethoprim+Sulfa | 1.25/23.75 | SxT25 | ≥ 30 | 29-26 | ≤ 25 | ≤ 0.12/2.3 | ≥ 0.5/9.5 |

a) Used for prophylaxis only (not treatment).

b) Trimethoprim+Sulfa predict susceptibility and resistance to Trimethoprim/Sulfamethoxazole and sulphonamides.

c) Oxacillin 1 µg is useful to screen for beta-lactamase negative meningococci with decreased susceptibility to Penicillin (chromosomal resistance). If the zone is < 10 mm (Oxacillin 1 µg), perform an MIC test for Penicillin.

d) Nalidixic acid is useful to screen for strains with reduced susceptibility to quinolones. If the zone is < 25 mm, control MIC's of Ciprofloxacin, Ofloxacin etc. Strains showing decreased susceptibility to Ciprofloxacin have been reported in Spain (9), Hong Kong (5, 7), USA (8) and Italy (10).

e) Mecillinam is used as a surrogate disk to screen for diminished Penicillin and Ampicillin susceptibility (6).

**References:**

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Zone diameter interpretative criteria and breakpoints according to CLSI (formerly NCCLS) (1,2) when testing gonococci are listed in the table below.

**Table 3.10-1 Interpretation for *Neisseria gonorrhoeae***

**GC Agar base and 1 % defined growth supplement. McFarland 0.5. Incubation at 35 °C ± 2 degrees in 5 % $CO_2$ for 20-24 hours.**

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| e) | Azithromycin | 15 µg | AZI15 | ≥ 24 | - | ≤ 23 | ≤ 1 | ≥ 2 |
| | Cefepime | 30 µg | FEP30 | ≥ 31 | - | - | ≤ 0.5 | - |
| d) | Cefixime | 5 µg | CFM.5 | ≥ 31 | - | - | ≤ 0.25 | - |
| | Cefotaxime | 30 µg | CTX30 | ≥ 31 | - | - | ≤ 0.5 | - |
| | Cefotetan | 30 µg | CFTTN | ≥ 26 | 25-20 | ≤ 19 | ≤ 2 | ≥ 8 |
| | Cefoxitin | 30 µg | CFO30 | ≥ 28 | 27-24 | ≤ 23 | ≤ 2 | ≥ 8 |
| | Cefpodoxime | 10 µg | CPD10 | ≥ 29 | - | - | ≤ 0.5 | - |
| d) | Ceftriaxone | 30 µg | CTR30 | ≥ 35 | - | - | ≤ 0.25 | - |
| | Cefuroxime | 30 µg | CXM30 | ≥ 31 | 30-26 | ≤ 25 | ≤ 1 | ≥ 4 |
| | Ciprofloxacin | 1 µg | CIPR1 | ≥ 32 | 31-21 | ≤ 20 | ≤ 0.06 | ≥ 1 |
| b) | Ciprofloxacin | 5 µg | CIPR5 | ≥ 41 | 40-28 | ≤ 27 | ≤ 0.06 | ≥ 1 |
| | Doxycycline | 30 µg | DOX30 | ≥ 38 | 37-31 | ≤ 30 | ≤ 0.25 | ≥ 2 |
| a) | Nalidixan (screen quinolones) | 30 µg | NAL30 | - | - | < 20 | decreased susceptibility to quinolones | |
| | Ofloxacin | 5 µg | OFL.5 | ≥ 31 | 30-25 | ≤ 24 | ≤ 0.25 | ≥ 2 |
| c) | Oxacillin (screen penicillin) | 1 µg | OXA.1 | ≥ 12 | - | - | ≤ 0.06 | - |
| d) | Penicillin | 10 U | PEN10 | ≥ 47 | 46-27 | ≤ 26 | ≤ 0.06 | ≥ 2 |
| | Spectinomycin | 200 µg | SPECT | ≥ 23 | 22-20 | ≤ 19 | ≤ 32 | ≥ 128 |
| | Tetracyclines | 30 µg | TET30 | ≥ 38 | 37-31 | ≤ 30 | ≤ 0.25 | ≥ 2 |

a) Nalidixic acid is useful to detect strains with reduced susceptibility to quinolones, however, strains of gonococci susceptible to Nalidixan acid and resistant to Ciprofloxacin were detected in the UK (5). Test both Nalidixan and Ciprofloxacin.

b) Ciprofloxacin resistant gonococci are presumably resistant to all fluoroquinolones.

c) Oxacillin 1 µg Neo-Sensitabs is useful to detect beta-lactamase negative gonococci, with decreased susceptibility to Penicillin (chromosomal resistance).

d) A positive beta-lactamase test predicts resistance to Penicillin, Ampicillin, Amoxycillin, Ticarcillin and Piperacillin. Emergence in Japan of beta-lactamase negative strains, resistant to Penicillin (MIC 2-8 µg/ml) and decreased susceptibility to Cefixime (MIC ≥ 0.5 µg/ml) and Ceftriaxone (MIC ≥ 0.125 µg/ml). They contain fragments of PBP-2 from *N. cinerea* and *N. perflava* (5).

e) Patients treated with Azithromycin for Chlamydia cannot be assumed to have been adequately treated for gonococcal infection. According to UK Department of Health Azithromycin should not be used to treat gonorrhoea (6).

f) Multidrug-resistant gonococci with reduced susceptibility to 3rd generation cephalosporins found in Greece (Tzelepi 7).

Unemo et al (8) indicates that the first N. gonorrhoeae strain highly resistant to Ceftriaxone and Cefixime was isolated in Japan. A similar strain was isolated in France. F89 has a Cefixime MIC of 4

µg/ml and Ceftriaxone MIC of 1 to 2 µg/ml. The strain was susceptible to Spectinomycin, but N. gonorrhoeae appears to be emerging as a superbug.

Katz et al (9) reports the first isolate of N. gonrrhoeae with high level resistance to Azithromycin, identified in the United States. The emergence of both cephalosporin and macrolide resistance would severely limit our treatment options for gonorrhoea.

Unemo et al (10) describe a high level Spectinomycin-resistant (MIC > 1.024 µg/ml) N. gonorrhoeae strain from Norway with a novel resistance mechanism. The isolate was susceptible to Cetriaxone and Cefixime.

**References:**

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# EUCAST-and CLSI potency NEO-SENSITABS™

## Interpretation Zones and MIC Breakpoints according to CLSI



Zone diameter interpretative criteria and MIC breakpoints when testing campylobacter are listed in the table below. The CLSI (formerly NCCLS) has established MIC breakpoints for Erythromycin, Ciprofloxacin, Tetracycline and Doxycycline (1). No zone diameter interpretative standards from CLSI are available for campylobacter so far.

Not more than 9 Neo-Sensitabs should be placed on a 150 mm agar plate or 4 Neo-Sensitabs on a 90-100 mm plate.

The plates are incubated at 36-37°C in Campylobacter atmosphere + catalysator for 42 to 48 hours, or at 42 °C for 20-24 hours, and the inhibition zones measured to the nearest mm.

**Table 3.11-1 Interpretation for *Campylobacter* spp.**

**Mueller-Hinton agar with 5 % blood added. McFarland 1.0. Incubation in Campylobacter atmosphere + catalysator at 36 °C for 42-48 hours or at 42 °C for 20-24 hours.**

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| | Azithromycin | 15 µg | AZI15 | ≥ 22 | 21-18 | ≤ 17 | ≤ 1 | ≥ 4 |
| | Amoxycillin | 25 µg | AMX25 | ≥ 28 | 27-24 | ≤ 23 | ≤ 2 | ≥ 8 |
| | Amoxycillin+Clavulanate | (20+10) µg | | ≥ 23 | 22-20 | ≤ 19 | ≤ 2 | ≥ 8 |
| | Ampicillin | 10 µg | AMP10 | ≥ 22 | 21-19 | ≤ 18 | ≤ 2 | ≥ 8 |
| | Cephalothin (ID) | 30 µg | CEP30 | - | - | < 14 (ID) | - | > 32 |
| | Chloramphenicol | 30 µg | CLR30 | ≥ 24 | 23-21 | ≤ 20 | ≤ 4 | ≥ 16 |
| | Ciprofloxacin (6,7) | 1 µg | CIPR1 | ≥ 18 | 14-17 | ≤ 13 | ≤ 1 | ≥ 4 |
| b) | Ciprofloxacin (6,7) | 5 µg | CIPR5 | ≥ 25 | 24-21 | ≤ 20 | ≤ 1 | ≥ 4 |
| | Doxycycline | 30 µg | DOX30 | ≥ 25 | 24-21 | ≤ 20 | ≤ 2 | ≥ 8 |
| b) | Erythromycin (6,7) | 15 µg | ERY15 | ≥ 16 | 15-13 | ≤ 12 | ≤ 8 | ≥ 32 |
| | Erythromycin (10) | 15 µg | ERY15 | ≥ 23 | - | < 23 | ≤ 1 | > 1 |
| | Gentamicin | 10 µg | GEN10 | ≥ 16 | 15 | ≤ 14 | ≤ 4 | ≥ 16 |
| | Imipenem | 10 µg | IMI10 | ≥ 26 | 25-23 | ≤ 22 | ≤ 1 | ≥ 2 |
| | Meropenem | 10 µg | MRP10 | ≥ 26 | 25-23 | ≤ 22 | ≤ 1 | ≥ 2 |
| a) | Nalidixan | 30 µg | NAL30 | | | | | |
| | identification (ID) | | | - | - | < 14 (ID) | - | > 128 |
| | screen quinolones | | | - | - | < 18 | - | ≥ 32 |
| | Tetracyclines | 30 µg | TET30 | ≥ 25 | 24-21 | ≤ 20 | ≤ 4 | ≥ 16 |

ID = for identification purposes only.

a) Strains resistant to Nalidixic acid show a decreased susceptibility to fluoroquinolones.

- For *Campylobacter* spp. the absence of zone of inhibition around beta-lactams, aminoglycosides, macrolides or quinolones indicates high level resistance.

b) According to CLSI, strains showing no zone of inhibition with Ciprofloxacin and Erythromycin should be reported as resistant to these antibiotics.

**QC ranges for Campylobacter jejuni ATCC 33560 (9)**

| Neo-Sensitabs™ | Potency | Zone diameter in mm. |
|---|---|---|
| Ciprofloxacin | 5 µg | 32-45 mm |
| Erythromycin | 15 µg | 26-38 mm |
| Nalidixic acid | 30 µg | 25-34 mm |
| Tetracycline | 30 µg | 29-39 mm |

**Note**: The proposed zone diameters are for 24-hour readings only
Lehtopolku et al. (10) recommend a lower MIC breakpoint and larger zones of inhibition for Erythromycin and Campylobacter. Erythromycin resistant strains (zone < 23mm) were uniformly resistant.
Perez Pomata et al. (11) conclude that the diffusion method is adequate for testing Erythromycin and Ciprofloxacin against isolates of *Campylobacter jejuni*.

Gaudreau (12) has published studies about the disk diffusion method for Erythromycin and Ciprofloxacin susceptibility testing of campylobacter spp. Here results match the CLSI Erythromycin and Ciprofloxacin disk diffusion method standardization: all isolates without zone of inhibition around Erythromycin 15 µg and Ciprofloxain 5 µg disks were resistant to macrolides and Ciprofloxacin.

**References:**

1) CLSI: Methods for Antimicrobial Dilution and Disk Susceptibility Testing of infrequently isolated fastidious bacteria. Approved Guideline.Suppl **M45-A**, 2006.
2) Vanhoof R. et al: Disk sensitivity testing for Campylobacter jejuni. Eur. J. Clin. Microbiol., **3**, 160-162, 1984.
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4) Lucero C. et al: *Campylobacter* spp.: Comparison of disc diffusion and agar dilution methods for susceptibility testing. ICAAC 2003, Presentation D-238.
5) McDermott P.F. et al: Development of a standardized susceptibility test for campylobacter with Q.C. ranges for Ciprofloxacin, Doxycycline, Erythromycin, Gentamicin and Meropenem. Microb. Drug Resist., **10**, 124-131, 2004.
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10) Lehtopolku et al.: Antimicrobial Susceptibilities of Multidrug Resistant *C. jejuni* and *C. coli* strains: in vitro activity of 20 antimicrobial agents. Antimicr. Ag. Chemother., 54, 1232-1236, 2010
11) Perez Pomata MT et al.: Susceptibility of *C. jejuni* Against Erythromycin and Ciprofloxacin by the diffusion method (Spanish). Presentation 033, SEIMC 2010, Barcelona.
12) Gaudreau C: Disk diffusion method for Erythromycin and Ciprofloxacin susceptibility testing of C. jejuni and C. coli. J. Clin. Microbiol. **51**, 380, 2013.

Zone diameter interpretative criteria and MIC breakpoints when testing *Helicobacter pylori* are listed in the table below. Only breakpoints for Clarithromycin are available from CLSI (formerly NCCLS) (1). Other MIC breakpoints or zone diameter interpretative standards for *Helicobacter pylori* have not yet been established by CLSI.

**Table 3.12-1 Interpretation for *Helicobacter pylori***

**Mueller-Hinton agar with 5-10 % blood added. McFarland 3 or 4. Incubation at 35-37 °C in a microaerophilic atmosphere (Campylobacter) for 72 hours.**

| | | | Zone diameter in mm | | | Break-points MIC µg/ml | |
|---|---|---|---|---|---|---|---|
| **NEO-SENSITABS** | **POTENCY** | **CODE** | **S** | **I** | **R** | **S** | **R** |
| Ampicillin | 10 µg | AMP10 | ≥ 26 | 25-21 | ≤ 20 | ≤ 0.5 | >1 |
| Azithromycin | 15 µg | AZI15 | ≥ 26 | 25-21 | ≤ 20 | ≤ 0.25 | ≥ 1 |
| Amoxycillin (use Ampicillin 10 µg) | | | | | | | |
| Ciprofloxacin | 5 µg | CIPR5 | ≥ 26 | 25-23 | ≤ 22 | ≤ 0.5 | ≥ 1 |
| Clarithromycin | 15 µg | CLA15 | ≥ 26 | 25-21 | ≤ 20 | ≤ 0.25 | ≥ 1 |
| Doxycycline | 30 µg | DOX30 | ≥ 30 | 29-26 | < 26 | ≤ 1 | >1 |
| Erythromycin | 15 µg | ERY15 | ≥ 20 | 19-17 | ≤ 16 | ≤ 1 | ≥ 4 |
| Levofloxacin | 5 µg | LEVOF | ≥ 26 | - | < 26 | ≤ 0.5 | ≥ 1 |
| Metronidazole | 16 µg | MTR16 | ≥ 26 | 26-23 | ≤ 22 | ≤ 8 | ≥ 16 |
| Moxifloxacin | 5 µg | MOXIF | ≥ 26 | - | < 26 | ≤ 0.5 | ≥ 1 |
| Rifampicin | 5 µg | RIF.5 | ≥ 21 | - | < 21 | - | - |
| Tetracyclines | 30 µg | TET30 | ≥ 30 | 29-26 | < 26 | ≤ 1 | >1 |

Because of the increasing resistance of *H. pylori* against Metronidazole and Clarithromycin, alternative regimens including newer fluoroquinolones have been suggested. Bogaerts et al. (8) showed that *H. pylori* resistance to fluoroquinolones is occurring at high frequency in Belgium and consequently the susceptibility of the infecting isolates to fluoroquinolones should be determined before administering these agents.

Rasmussen et al (10) found an excellent correlation between E-test and Neo-Sensitabs for Metronidazole and Ciprofloxacin and acceptable correlation for Ampicillin, Tetracycline and Clarithromycin.

Wüppenhorst et al. (11) isolated one strain of H. pylori being resistant to Metronidazole (MIC ≥ 256 µg/ml), Clarithromycin (MIC 16 µg/ml), Levofloxacin (MIC ≥ 32 µg/ml) and Rifampicin (MIC ≥ 32 µg/ml), but susceptible to Amoxycillin (MIC 0.05 µg/ml). Multiresistant clinical H. pylori isolates exist in Germany and will probably increase in the future.

**References:**


1) CLSI: Performance Standards for Antimicrobial Susceptibility Testing. 23rd Inf. Suppl. **M100-S23**, 2013.
2) Huaxiang X. et al: Standardisation of Disk Diffusion Test and clinical significance for susceptibility testing of Metronidazole against *Helicobacter pylori*. Antimicr. Ag. Chemother., **38**, 2357-2361, 1994.
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9) Henriksen T.H. et al: A simple method for determining Metronidazole resistance of H. pylori. J. Clin. Microbiol., **35**, 1424-26, 1997.
10) Rasmussen L. et al: Comparison of the E-test and the Neo-Sensitabs method for antimicrobial susceptibility testing of H. pylori. ECCMID poster P-1179, Barcelona 2008.
11) Wüppenhorst N. et al: Isolation and eradication of clinical isolate of Helicobacter *pylori* resistant to five antimicrobials in Germany. J. Antimicr. Chemother. **66**, 222-223, 2011.

Zone diameter interpretative criteria and MIC breakpoints according to CLSI (formerly NCCLS) (1) when testing *Vibrio cholerae* are listed in the table below.

**Table 3.13-1 Interpretation for *Vibrio cholerae***

**Mueller-Hinton agar. Inoculum McFarland 0.5. Incubation 35 °C ± 2 degrees ambient air for 16-18 hours.**

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | Zone diameter in mm I | Zone diameter in mm R | Break-points MIC µg/ml S | Break-points MIC µg/ml R |
|---|---|---|---|---|---|---|---|---|
| a) | Ampicillin | 10 µg | AMP10 | ≥ 17 | 16-14 | ≤ 13 | ≤ 8 | ≥ 32 |
| | Tetracyclines | 30 µg | TET30 | ≥ 19 | 18-15 | ≤ 14 | ≤ 4 | ≥ 16 |
| | Sulphonamides | 240 µg | SULFA | ≥ 23 | 22-20 | ≤ 19 | ≤ 100 | ≥ 350 |
| | Trimethoprim+Sulfa | 1.25+23.75µg | SxT25 | ≥ 16 | 15-11 | ≤ 10 | ≤ 2/38 | ≥ 8/152 |
| | Chloramphenicol | 30 µg | CLR30 | ≥ 18 | 17-13 | ≤ 12 | ≤ 8 | ≥ 32 |
| | Doxycycline | 30 µg | DOX30 | ≥ 16 | 15-13 | ≤ 12 | ≤ 4 | ≥ 16 |
| b) | Nalidixan | 30 µg | NAL30 | - | - | < 15 | - | reduced susceptibility to quinolones |
| | - screening quinolones | | | | | | | |
| | Azithromycin | 15µg | AZI15 | | | | | |

a) Results for Ampicillin are used to predict susceptibility to Amoxycillin.

b) Strains resistant to Nalidixic acid show a decreased susceptibility to fluoroquinolones.

**References:**


1) CLSI: Performance Standards for Antimicrobial Susceptibility Testing. 23rd Inf. Suppl. **M100-S23**, 2013.
2) Mukhopadhyay A.K et al: Emergence af Fluoroquinolone resistance in strains of *V. cholerae* isolated from hospitalized patients with acute diarrhoea in Calcutta, India. Antim. Ag. Chemother., **42**, 206-207, 1998.
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4) Lai-King Ng et al: Can E-test be used to determine *Vibrio cholerae* susceptibility to Erythromycin? Antimicr. Ag. Chemother., **47**, 1479-80, 2003.

Organisms recognized as virulent or commonly resistant, should be considered for testing. These include the *Bacteroides fragilis* group, *Prevotella* and *Porphyromonas* spp., *Clostridium perfringens*, *Cl. ramosum, Cl. septicum* and *Cl. difficile*.

The recommended procedure for susceptibility testing of anaerobes by the diffusion method is the following:

- Use supplemented Brucella blood agar, it supports good growth for essentially all anaerobes. Brucella agar base is supplemented with 5 µg/ml haemin, 5% defibrinated sheep blood and 1 µg/ml vitamin K1 (haemin and vitamin K1 may be added before sterilisation).
- Direct suspension of colonies in broth to achieve a turbidity equivalent to a 1.0 McFarland standard ($3x10^8$ CFU/ml). Streak the surface of the agar with a cotton swab.
- Allow the inoculated plate to remain at room temperature of ≤25°C (5-10 min.) until the surface of the agar looks dry. For some fastidious isolates that do not grow on control plates, pre-reduction of plates in an anaerobic environment may be necessary. Apply Neo-Sensitabs tablets.
- Invert the inoculated plate and incubate at 35°C in an anaerobic jar or alternative anaerobic environment, for 24-48 hours.

Zone diameter interpretative standards are correlated to the MIC breakpoints recommended by the CLSI for anaerobic bacteria (1), but zone diameter interpretative standards have not yet been established by CLSI.

**Table 3.14-1 Interpretation for Anaerobes**

**Supplemented Brucella Blood agar. Inoculum: McFarland 1.0. Incubation at 35 °C in anaerobic environment for 24-48 hours.**

| | NEO-SENSITABS | | | Zone diameter in mm | | | Break-points MIC µg/ml | |
|---|---|---|---|---|---|---|---|---|
| | | | | S | I | R | S | R |
| b) | Amoxycillin+Clav. | 20+10 µg | AMC30 | ≥ 24 | 23-21 | ≤ 20 | ≤ 4 / 2 | ≥ 16 / 8 |
| | Cefoxitin | 30 µg | CFO30 | ≥ 16 | 15-12 | ≤ 11 | ≤ 16 | ≥ 64 |
| | Chloramphenicol | 30 µg | CLR30 | ≥ 23 | 22-20 | ≤ 19 | ≤ 8 | ≥ 32 |
| | Clindamycin | 2 µg | CLI.2 | ≥ 15 | 14-10 | No zone | ≤ 2 | ≥ 8 |
| | Doxycycline | 30 µg | DOX30 | ≥ 22 | 21-19 | ≤ 18 | ≤ 4 | ≥ 16 |
| | Doripenem | 10 µg | DOR10 | | | | ≤ 2 | ≥ 8 |
| a) | Ertapenem | 10 µg | ERTAP | ≥ 23 | 22-20 | ≤ 19 | ≤ 4 | ≥ 16 |
| | Fucidin | 10 µg | FUC10 | ≥ 18 | - | ≤ 18 | ≤ 2 | ≥ 4 |
| a) | Imipenem | 10 µg | IMI10 | ≥ 22 | 21-18 | ≤ 17 | ≤ 4 | ≥ 16 |
| c) | Imipenem+EDTA | 10+750 µg | IM10E | Detection of metallo-β-lactamases | | | | |
| | Linezolid | 30 µg | LINEZ | ≥ 21 | - | < 21 | ≤ 4 | > 4 |
| c | Meropenem | 10 µg | MRP10 | ≥ 22 | 21-18 | ≤ 17 | ≤ 4 | ≥ 16 |
| b) | Metronidazole | 16 µg | MTR16 | ≥ 22 | 21-19 | ≤ 18 | ≤ 8 | ≥ 32 |
| | Clostridium difficile | | | ≥ 28 | - | - | ≤ 2 | - |
| | Moxifloxacin | 5 µg | MOXIF | ≥ 23 | 22-20 | ≤ 19 | ≤ 2 | ≥ 8 |
| | Penicillin (Ampicillin) | 10 U | PEN10 | ≥ 25 | 24-21 | ≤ 20 | ≤ 0.5 | ≥ 2 |
| | Piperacillin+Tazobactam | 100+10µg | PI+TZ | ≥ 24 | 23-21 | ≤ 20 | ≤ 32 / 4 | ≥ 128 / 4 |
| | Teicoplanin | 30 µg | TPN30 | ≥ 18 | - | - | ≤ 4 | - |
| | Ticarcillin+Clavulanate | 75+10 µg | TIM85 | ≥ 24 | 23-21 | ≤ 20 | ≤ 32 / 2 | ≥ 128 / 2 |
| | Tigecycline | 15 µg | TIG15 | ≥ 22 | 21-16 | ≤ 14 | ≤ 2 | ≥ 8 |
| | Vancomycin | 30 µg | VAN30 | ≥ 18 | - | - | ≤ 4 | - |

a) The carbapenems show typically MIC's of ≤ 0.12 µg/ml against *B. fragilis* (inhibition zones ≥ 30 mm). Strains showing MIC's of 2-4 µg/ml (zones: 22-26 mm) may sometimes conduct to therapeutic failure (increased expression of cfiA (6)).

b) The SFM recommends Metronidazole Neo-Sensitabs for sensitivity testing of anaerobes, due to stability problems with paper disks.
Baines et al (8) found that Cl. difficile ribotype 001 had reduced susceptibility to Metronidazole (MIC 3.5 µg/ml). To detect these strains, the susceptible MIC breakpoint should be lowered to S ≤2 µg/ml (zone ≥ 28mm (S)).

c) See description of technique for the detection of metallo-β-lactamases in "Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs™".

d) Peptococcus/Bacteroides spp: If resistant to Erythromycin, but susceptible to Clindamycin, report resistant to Clindamycin ($MLS_B$ phenotype).

Wybo et al (7) in a Belgian study found that the prevalence of cfiA resistance gene was 6.7% in B. fragilis. Using the CLSI breakpoints (4/16 µg/ml), 3 of the 9 strains would be reported as susceptible to Meropenem, but with the EUCAST breakpoints (2/8 µg/ml) no false susceptible were obtained.

Quality Control Limits for anaerobes

Suppl. Brucella Blood Agar
Inoc. McF 1.0 Anaerobic incubation

| Neo-Sensitabs | | CODE | Bact. Fragilis ATCC 25285 | MIC µg/ml | B.thetaiotaomicron ATCC29741 | MIC µg/ml |
|---|---|---|---|---|---|---|
| Amoxycillin+Clav. | 20+10 µg | AMC30 | 30-38 mm | 0.5 | 33-38 mm | 0.5 |
| Cefoxitin | 30 µg | CFO30 | 21-28 mm | 8 | 17-24 mm | 16 |
| Cloramphenicol | 30 µg | CLR30 | 26-32 mm | 8 | 26-32 mm | 16 |
| Clindamycin | 2 µg | CLI2 | 14-20 mm | 1 | 14-20 mm | 4 |
| Imipenem | 10 µg | IMI10 | 32-40 mm | 0.06 | 32-40 mm | 0.12 |
| Meropenem | 10 µg | MRP10 | 29-35 mm | 0.12 | 29-35 mm | 0.12/0.25 |
| Metronidazole | 16 µg | MTR16 | 29-36 mm | 0.5 | 30-36 mm | 1 |

Goldstein et al (9) indicates that susceptibility testing of anaerobes is performed in a minority of laboratories. Beta-lactamase is the most common resistance mechanism. Particularly in Bacteroides fragilis group are common but also found in Clostridia, Porphyromonas and Fusobacterium. Carbapenemases (metallo-beta-lactamases) are found in the B. fragilis group and some Prevotella spp. Metronidazole resistance has been reported worldwide in the B.fragilis group.
Fluoroquinolone resistance (moxifloxacin) is found in the B. fragilis group and in C. difficile.

**References:**

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2) Edwards R.et al: Mechanism responsible for reduced susceptibility to Imipenem in *Bacteroides fragilis*. J. Antimicrob. Chemother., **38**, 941-951, 1996.
3) Cormican M.G. et al: False resistance to Metronidazole by E-test, among anaerobic bacteria. Diagn. Microbiol. Infect. Dis., **24**, 117-119, 1996.
4) Fang H. et al: Detection of Imipenem-resistant and Metronidazole-resistant *Bact. fragilis* group strains in fecal samples. Clin. Microbiol. Infect., **5**, 753-8, 1999.
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8) Baines S. et al: Emergence of reduced susceptibility to Metronidazole in Clostridium difficle. ECCMID, abstract O463, april, 2008, Barcelona.
9) Goldstein EJC et al: Resistance trends in antimicrobial susceptibility of anaerobic bacteria. Clin. Microbiol. Newsletter. **33**, 1-15, 2011.

Only Fluconazole and Voriconazole are included in the M44-A guideline from CLSI (formerly NCCLS)(1) and interpretation zones are only available for Fluconazole so far. When MIC breakpoints are available from CLSI (2) they are used in the table below.

**Table 13.5-1 Interpretation for Yeasts**

**Mueller-Hinton Glucose Methylene Blue agar. Inoculum McFarland 0.5 undiluted. Incubation at 35 °C ± 2 °C ambient air for 20-24 hours. MICs according to M27-S3 (2007).**

| | | | Zone diameter in mm | | | Break-points MIC µg/ml | |
|---|---|---|---|---|---|---|---|
| **NEO-SENSITABS** | **POTENCY** | **CODE** | **S** | **I** | **R** | **S** | **R** |
| Amphotericin B | 10 µg | AMPHO | ≥ 15 | 14-10 | < 10 | ≤ 1 | ≥ 2 |
| Caspofungin *) (24) | 5 µg | CASP5 | | | | | |
| C. albicans, C. tropicalis | | | ≥ 17 | 16-15 | ≤ 14 | ≤ 0.25 | ≥ 1 |
| C. parapsilosis,* C. guilliermondii | | | ≥ 13 | 12-11 | ≤ 10 | ≤ 2 | > 2 |
| C. glabrata, C. krusei | | | ≥ 16 | - | - | ≤ 0.5 | - |
| Fluconazole **) (27) | 25 µg | FLUCZ | | | | | |
| C. albicans, C. tropicalis, C. parapsilosis | | | ≥ 17 | 16-14 | ≤ 13 | ≤ 2 | ≥ 8 |
| C. glabrata | | | - | ≥ 15 (SDD) | ≤ 14 | ≤ 32 (SDD) | ≥ 64 |
| Itraconazole (11) | 10 µg | ITRAC | ≥ 23 | 22-14 (SDD) | ≤ 13 | ≤ 0.12 | > 0.5 |
| Ketoconazole | 15 µg | KETOC | ≥ 28 | 27-21 | ≤ 20 | ≤ 0.12 | ≥ 0.5 |
| Posaconazole (9,12) | 5 µg | POSAC | ≥ 17 | 16-14 (SDD) | ≤ 13 | ≤ 1 | > 2 |
| Voriconazole (23) | 1 µg | VOR.1 | | | | | |
| C. albicans, C. tropicalis, C. parapsilosis | | | ≥ 17 | 16-15 | ≤ 14 | ≤ 0.12 | ≥ 1 |
| C. krusei | | | ≥ 15 | 14-13 | ≤ 12 | ≤ 0.5 | ≥ 2 |
| C. glabrata | | | ≥ 16 | - | ≤ 15 | ≤ 0.5 | > 0.5 |

*) Tentative. There is cross-resistance between Caspofungin and the other echinocandins: Anidulafungin and Micafungin (19).

**) *C. krusei* should be reported as resistant to Fluconazole (no matter the zone).

For further information on Susceptibility Testing of Yeasts, see Neo-Sensitabs User's Guide.

**Table 13.5-2 Mold Disk Diffusion Testing (14, 17,21)**

**Mueller-Hinton Plain. Incubation for 16-24 hours (zygomycetes), 24 hours (Aspergillus), 48 hours (other spp.). Temperature: 35-37°C**

**Inoculum:**

Prepare a suspension of sporulating colonies in 0.85% saline, add 1 drop of Tween 20. Allow heavy particles to settle for 3-5 minutes and the upper suspension is treated for 15 seconds in a vortex mixer. The density of the suspension is read on a spectrophotometer at 530 mm wave length and the optical density adjusted at 0.09 to 0.13 for Aspergillus.

| NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|
| Amphotericin B (zygomycetes only) | 10 µg | AMPHO | ≥ 15 | - | - | ≤ 1 | - |
| Caspofungin | 5 µg | CASP5 | ≥ 17 | - | - | ≤ 1 | - |
| Itraconazole | 10 µg | ITRAC | ≥ 17 | - | - | ≤ 1 | ≥ 2 |
| Posaconazole | 5 µg | POSAC | ≥ 23 | - | - | ≤ 0.25 | ≥ 0.5 |
| Voriconazole | 1 µg | VOR.1 | ≥ 17 | - | - | ≤ 1 | ≥ 2 |

**Note:** The base medium should not be supplemented with neither 2% glucose nor 0.5% methylene blue dye.

Itraconazole susceptible strains should be reported as susceptible to both, Posaconazole and Voriconazole.

### Interpretation table for Local treatment

In local treatment of fungal infections, a high concentration of antifungal is placed at site of the infection. Consequently other MIC breakpoints and zone interpretations should be used in those cases.

| Local Treatment<br>MH Glucose Methylene Blue Agar or Shadomy<br>McFarland 0.5 inoculum | | | |
|---|---|---|---|
| **Susceptible** | ≥ 20 mm | ≥ 15 mm | ≥ 10 mm |
| **Intermediate** | 12-19 mm | 10-14 mm | - |
| **Resistant** | ≤ 11 mm | no zone | no zone |
| | Ciclopirox<br>Clotrimazole<br>Econazole<br>Fluconazole<br>Isoconazole<br>Ketoconazole<br>Miconazole<br>Tioconazole<br>Terbinafine | Natamycin<br>Nystatin<br>Itraconazole | Griseofulvin |

Fluorocytosine cannot be tested on MH-agar (antagonists), but has to be tested on Shadomy agar or similar.

**Table 13.5-3 Quality Control Zone Diameters (mm) Ranges**

**Mueller-Hinton Glucose Methylene Blue agar. Inoculum McFarland 0.5 undiluted. Incubation at 35 °C ± 2 °C for 20-24 hours.**

| | | | Zone diameter in mm | | |
|---|---|---|---|---|---|
| **NEO-SENSITABS** | **POTENCY** | **CODE** | ***C. albicans* ATCC 90028** | ***C. parapsilosis* ATCC 22019** | ***C. krusei* ATCC 6258** |
| Amphotericin B | 10 µg | AMPHO | 20-27 | 22-29 | 18-25 |
| Fluconazole | 25 µg | FLUCZ | 28-39 | 22-33 | - |
| Itraconazole | 10 µg | ITRAC | 21-30 | 19-26 | 16-22 |
| Ketoconazole | 15 µg | KETOC | 31-42 | 35-45 | 22-29 |
| Voriconazole | 1 µg | VOR.1 | 31-42 | 28-37 | 23-31 |
| Caspofungin | 5 µg | CASP5 | 15-22 | 13-23 | 16-22 |
| Posaconazole | 5 µg | POSAC | 24-34 | 25-36 | 23-31 |

Carrillo-Munoz et al (30) determined Posaconazole susceptibility of clinical yeast isolates with Neo-Sensitabs and a microdilution method. Complete agreement between Posaconazole Neo-Sensitabs and the microdilution was 92.3 % after 24 hours incubation. The authors conclude that the Agar Diffusion with Posaconazole Neo-sensitabs can improve Posaconazole susceptibility testing due to its excellent correlation and reduced percentage of disagreements in comparison with microdilution testing.

**References:**

1) NCCLS. Methods for Antifungal Disk Diffusion Susceptibility Testing on Yeasts. Approved Guideline M44-A2, 2008.
2) NCCLS. Reference Methods for Broth Dilution Antifungal Susceptibility Testing of yeasts. Approved Standard,
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3) Vandenbossche I. et al: Susceptibility testing of Fluconazole by the NCCLS broth macrodilution method. E-Test and Disk diffusion for application in the routine laboratory. J. Clin. Microbiol, **40**, 918-921, 2002.
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The acceptable limits for quality control strains, using agars and inoculum according to CLSI (Kirby-Bauer) are seen in the tables below (1,2).

**Inoculum according to CLSI (Kirby-Bauer)**

| | | | Zone diameter in mm | | | | |
|---|---|---|---|---|---|---|---|
| **NEO-SENSITABS** | | **CODE** | ***E. coli* ATCC 25922** | ***S. aureus* ATCC 25923** | ***Ps. aeruginosa* ATCC 27853** | ***E. coli* ATCC 35218** | ***E.faecalis ATCC 29212*** |
| AMIKACIN | 30 µg | AMI30 | 19-26 | 20-26 | 18-26 | - | |
| AMOXYCILLIN | 25 µg | AMX25 | 21-26 | - | - | - | |
| AMOXYCILLIN+ CLAV. | 20+10 µg | AMC30 | 18-24 | 28-36 | - | 17-22 | |
| AMPICILLIN | 10 µg | AMP10 | 16-22 | 27-35 | - | 9 | |
| AMPICILLIN+ SULBACTAM | 10+10 µg | SAM20 | 19-24 | 29-37 | | 13-19 | |
| AZITHROMYCIN | 15 µg | AZI15 | - | 21-26 | - | - | |
| AZTREONAM | 30 µg | AZT30 | 28-36 | - | 23-29 | - | |
| CEFTOBIPROLE | 30 µg | CFBIP | 30-36 | 26-34 | 23-31 | - | |
| CEFAZOLIN | 30 µg | CFZ30 | 21-27 | 29-35 | - | - | |
| CEFEPIME | 30 µg | FEP30 | 31-37 | 23-29 | 24-30 | - | |
| CEFIXIME | 5 µg | CFM.5 | 23-27 | - | - | - | |
| CEFOTAXIME | 30 µg | CTX30 | 29-35 | 25-31 | 18-22 | - | |
| CEFOTAXIME | 5 µg | CTX5 | 25-31 | - | - | - | |
| CEFOXITIN | 30 µg | CFO30 | 23-29 | 23-29 | - | - | |
| CEFPODOXIME | 10 µg | CPD10 | 23-28 | 19-25 | - | - | |
| CEFTAROLINE | 30 µg | CPT30 | 26-34 | 26-35 | - | - | |
| CEFTAZIDIME | 30 µg | CAZ30 | 25-32 | 16-20 | 22-29 | - | |
| CEFTAZIDIME | 10 µg | CAZ10 | 23-29 | - | 21-27 | - | |
| CEFTIZOXIME | 30 µg | ZOX30 | 30-36 | 27-35 | 12-17 | - | |
| CEFTRIAXONE | 30 µg | CTR30 | 29-35 | 22-28 | 17-23 | - | |
| CEFUROXIME | 30 µg | CXM30 | 20-26 | 27-35 | - | - | |
| CEPHALOTHIN | 30 µg | CEP30 | 15-21 | 29-37 | - | - | |
| CHLORAMPHENICOL | 30 µg | CLR30 | 21-27 | 19-26 | - | - | |
| CIPROFLOXACIN | 5 µg | CIPR5 | 30-40 | 22-30 | 25-33 | - | |
| CLARITHROMYCIN | 15 µg | CLA15 | - | 26-32 | - | - | |
| CLINDAMYCIN | 2 µg | CLI.2 | - | 24-30 | - | - | |
| COLISTIN | 10 µg | CO.10 | - | - | - | - | |
| 2+18 hours prediffusion | | | 22-28 | - | 17-23 | - | |
| DALBAVANCIN | 60 µg | DAL60 | | | | | |
| 4+20 hours prediffusion | | | - | 20-26 | - | - | 19-25 |
| DAPTOMYCIN | 30 µg | DAPCa | - | - | - | - | |
| 2+18 hours prediffusion | | | - | 22-28 | - | - | |
| DORIPENEM | 10 µg | DOR10 | 27-35 | 33-42 | 28-35 | - | |
| DOXYCYCLINE | 30 µg | DOX30 | 18-24 | 23-29 | - | - | |
| ERTAPENEM | 10 µg | ETP10 | 29-36 | 24-31 | 13-21 | - | |
| ERYTHROMYCIN | 15 µg | ERY15 | - | 22-30 | - | - | |
| FOSFOMYCIN | 200 µg | FO200 | 22-30 | 25-33 | - | - | |
| FUCIDIN | 10 µg | FUC10 | - | 24-32 | - | - | |
| GATIFLOXACIN | 5 µg | GATIF | 30-37 | 27-33 | 20-28 | - | |

**Zone diameter in mm**

| NEO-SENSITABS | | CODE | *E. coli* ATCC 25922 | *S. aureus* ATCC 25923 | *Ps. aeruginosa* ATCC 27853 | *E. coli* ATCC 35218 | *E.faecalis ATCC 29212* |
|---|---|---|---|---|---|---|---|
| GENTAMICIN | 10 µg | GEN10 | 19-26 | 19-27 | 17-23 | - | |
| GENTAMICIN | 250 µg | | - | - | - | - | |
| ICLAPRIM | 5 µg | | 14-22 | 25-33 | - | - | |
| IMIPENEM | 10 µg | IMI10 | 26-32 | - | 20-28 | - | |
| KANAMYCIN | 30 µg | KAN30 | 17-25 | 19-26 | - | - | |
| LEVOFLOXACIN | 5 µg | LEVOF | 29-37 | 25-30 | 19-26 | - | |
| LINEZOLID | 30 µg | LINEZ | - | 25-32 | - | - | |
| MECILLINAM | 10 µg | MEC10 | 24-30 | - | - | - | |
| MEROPENEM | 10 µg | MRP10 | 28-34 | 29-37 | 27-33 | - | |
| MINOCYCLINE | 30 µg | MIN30 | 19-25 | 25-30 | - | - | |
| MOXIFLOXACIN | 5 µg | MOXIF | 28-35 | 28-35 | 17-25 | - | |
| MUPIROCIN | 10 µg | MUPIR | - | 21-26 | - | - | |
| MUPIROCIN | 200 µg | MP200 | - | 29-38 | - | - | |
| NALIDIXAN | 30 µg | NAL30 | 22-28 | - | - | - | |
| NETILMICIN | 30 µg | NET30 | 22-30 | 22-31 | 17-23 | - | |
| NITROFURANTOIN | 300 µg | NI300 | 20-25 | 18-22 | - | - | |
| NORFLOXACIN | 10 µg | NORFX | 28-35 | 17-28 | 22-29 | - | |
| NOVOBIOCIN | 5 µg | NOV.5 | - | 18-25 | - | - | |
| OFLOXACIN | 5 µg | OFL.5 | 29-33 | 24-28 | 17-21 | - | |
| OXACILLIN | 1 µg | OXA.1 | - | 18-24 | - | - | |
| PIPERACILLIN+TAZOBACTAM | 30+6 µg | PTZ36 | 21-27 | - | 23-29 | - | |
| PENICILLIN | 10U | PEN10 | - | 26-37 | - | - | |
| PIPERACILLIN | 100 µg | PIPRA | 24-30 | - | 25-33 | 12-18 | |
| PIPERACILLIN+ TAZOBACTAM | 100+10 µg | PI+TZ | 24-30 | 27-36 | 25-33 | 24-30 | |
| POLYMYXINS | 150 µg | CO150 | 19-24 | - | 20-25 | - | |
| QUINUPRISTIN/ DALFOPRISTIN | 15 µg | SYN15 | - | 21-28 | - | - | |
| RIFAMPICIN | 5 µg | RIF.5 | - | 26-34 | - | - | |
| RETAPAMULIN | 2 µg | RETA2 | - | 23-30 | - | - | |
| STREPTOMYCIN | 10 µg | STR10 | 12-20 | 14-22 | - | - | |
| SULPHONAMIDES | 240 µg | SULFA | 18-25 | 23-33 | - | - | |
| TEICOPLANIN | 30 µg | TPN30 | - | 15-21 | - | - | |
| 2+18 hours prediffusion | | | - | 22-28 | - | - | |
| TELITHROMYCIN | 15 µg | TEL15 | - | 24-30 | - | - | |
| TEMOCILLIN | 30 µg | TEMOC | 20-26 | - | - | 22-28 | |
| TETRACYCLINES | 30 µg | TET30 | 18-25 | 24-30 | - | - | |
| TICARCILLIN | 75 µg | TIC75 | 24-30 | - | 21-27 | 9 | |
| TICARCILLIN+ CLAVULANATE | 75+10 µg | TIM85 | 24-30 | 29-37 | 20-28 | 21-25 | |
| TIGECYCLINE | 15 µg | TIG15 | 20-27 | 20-25 | 9-13 | - | |
| TOBRAMYCIN | 10 µg | TOB10 | 18-26 | 19-29 | 20-26 | - | |

**Zone diameter in mm**

| NEO-SENSITABS | | CODE | *E. coli* ATCC 25922 | *S. aureus* ATCC 25923 | *Ps. aeruginosa* ATCC 27853 | *E. coli* ATCC 35218 | *E.faecalis ATCC 29212* |
|---|---|---|---|---|---|---|---|
| TRIMETHOPRIM | 5 µg | TRIM5 | 21-28 | 19-26 | - | - | |
| TRIMETHOPRIM+ SULFA | 1.25+23.75 µg | TR+SU | 23-29 | 24-32 | - | - | |
| VANCOMYCIN | 30 µg | VAN30 | - | 17-21 | - | - | |
| 2+18 hours prediffusion | | | - | 20-27 | - | - | |

| | | | ***K.pneumoniae* ATCC 700603** | | | | |
|---|---|---|---|---|---|---|---|
| AMOXYCILLIN+CLAV | 20+10 µg | AMC30 | 15-19 | | | | |
| AZTREONAM | 30 µg | AZT30 | 9-17 | | | | |
| CEFOTAXIME | 30 µg | CTX30 | 17-25 | | | | |
| CEFPODOXIME | 10 µg | CPD10 | 9-16 | | | | |
| CEFTAZIDIME | 30 µg | CAZ30 | 10-18 | | | | |
| CEFTRIAXONE | 30 µg | CTR30 | 16-24 | | | | |

# EUCAST-and CLSI potency NEO-SENSITABS™

## Interpretation Zones and MIC Breakpoints according to CLSI

## Control Limits on Mueller-Hinton with Blood and ± Supplements for Fastidious Organisms

**Inoculum according to CLSI (Kirby-Bauer)**

| NEO-SENSITABS | | CODE | Zone diameter in mm | | | |
|---|---|---|---|---|---|---|
| | | | ***Haemoph. influenzae*** **ATCC 49247** | ***Haemoph. influenzae*** **ATCC 49766** | ***Neisseria gonorrhoeae*** **ATCC 49226** | ***Streptoc. pneumoniae*** **ATCC 49619** |
| AMOXYCILLIN | 30 µg | AMOXY | | | | 36-42 |
| AMOXYCILLIN+ CLAVULANATE | 20+10 µg | AMC30 | 15-23 | - | - | - |
| AMPICILLIN | 10 µg | AMP10 | 13-21 | - | - | 30-36 |
| AMPICILLIN | 2.5 µg | AMP.L | 9-14 | - | - | 25-31 |
| AMPICILLIN+ SULBACTAM | 10+10 µg | SAM20 | 14-22 | - | - | - |
| AZITHROMYCIN | 15 µg | AZI15 | 13-21 | - | 27-36 | 19-25 |
| AZTREONAM | 30 µg | AZT30 | 30-38 | - | - | - |
| CEFTOBIPROLE | 30 µg | CFBIP | 28-36 | - | - | 32-39 |
| CEFEPIME | 30 µg | FEP30 | 25-31 | - | 37-46 | 31-37 |
| CEFIXIME | 5 µg | CFM.5 | 25-33 | - | 37-45 | 16-23 |
| CEFOTAXIME | 30 µg | CTX30 | 31-39 | - | 38-48 | 31-39 |
| CEFOTAXIME | 5 µg | CTX5 | | | | 28-34 |
| CEFOXITIN | 30 µg | CFO30 | - | - | 33-41 | - |
| CEFPODOXIME | 10 µg | CPD10 | 25-31 | - | 35-43 | 28-34 |
| CEFTAROLINE | 30 µg | CPT30 | 29-39 | - | - | 31-41 |
| CEFTAZIDIME | 30 µg | CAZ30 | 27-35 | - | 35-43 | - |
| CEFTIZOXIME | 30 µg | ZOX30 | 29-39 | - | 42-51 | 28-34 |
| CEFTRIAXONE | 30 µg | CTR30 | 31-39 | - | 39-51 | 30-35 |
| CEFUROXIME | 30 µg | CXM30 | - | 28-36 | 33-41 | 28-34 |
| CEPHALOTHIN | 30 µg | CEP30 | - | - | - | 26-32 |
| CHLORAMPHENICOL | 30 µg | CLR30 | 31-40 | - | - | 23-27 |
| CIPROFLOXACIN | 5 µg | CIPR5 | 34-42 | - | 48-58 | - |
| CIPROFLOXACIN | 1 µg | CIPR1 | -- | - | 38-48 | - |
| CLARITHROMYCIN | 15 µg | CLA15 | 11-17 | - | - | 25-31 |
| CLINDAMYCIN | 2 µg | CLI.2 | - | - | - | 19-25 |
| DOXYCYCLINE | 30 µg | DOX30 | - | - | - | 25-34 |
| DORIPENEM | 10 µg | DOR10 | 21-31 | - | - | 30-38 |
| ERTAPENEM | 10 µg | ETP10 | 20-28 | 27-33 | - | 28-35 |
| ERYTHROMYCIN | 15 µg | ERY15 | | | | 25-30 |
| GATIFLOXACIN | 5 µg | GATIF | 33-41 | - | 45-56 | 24-31 |
| IMIPENEM | 10 µg | IMI10 | 21-29 | - | - | 34-42 |
| LEVOFLOXACIN | 5 µg | LEVOF | 32-40 | - | - | 20-25 |
| LINEZOLID | 30 µg | LINEZ | - | - | - | 25-34 |
| MEROPENEM | 10 µg | MRP10 | 20-28 | - | - | 28-35 |
| MOXIFLOXACIN | 5 µg | MOXIF | 31-39 | - | - | 25-31 |
| NITROFURANTOIN | 300 µg | NI300 | - | - | - | 23-29 |
| NORFLOXACIN | 10 µg | NORFX | - | - | - | 15-21 |
| OFLOXACIN | 5 µg | OFL.5 | 31-40 | - | 43-51 | 16-21 |
| OXACILLIN | 1 µg | OXA.1 | - | - | - | 9-14 |
| PIPERACILLIN+TAZOBACTAM | 30+6 µg | PTZ36 | - | - | - | 26-32 |
| PENICILLIN | 10U | PEN10 | - | - | 26-34 | 24-30 |
| PIPERACILLIN+ TAZOBACTAM | 100+10 µg | PI+TZ | 33-38 | - | - | - |

| NEO-SENSITABS | | CODE | Zone diameter in mm | | | |
|---|---|---|---|---|---|---|
| | | | ***Haemoph. influenzae*** **ATCC 49247** | ***Haemoph. influenzae*** **ATCC 49766** | ***Neisseria gonorrhoeae*** **ATCC 49226** | ***Streptoc. pneumoniae*** **ATCC 49619** |
| QUINUPRISTIN/ DALFOPRISTIN | 15 µg | SYN15 | 15-21 | - | - | 19-24 |
| RETAPAMULIN | 2 µg | RETA2 | - | - | - | 13-19 |
| RIFAMPICIN | 30 µg | RIF.5 | 22-30 | - | - | 25-30 |
| SPECTINOMYCIN | 200 µg | SPECT | - | - | 26-35 | - |
| TELITHROMYCIN | 15 µg | TEL15 | 17-23 | - | - | 27-33 |
| TETRACYCLINES | 30 µg | TET30 | 14-22 | - | 30-42 | 27-31 |
| TIGECYCLINE | 15 µg | TIG15 | 23-31 | - | 30-40 | 23-29 |
| TRIMETHOPRIM+ SULFA | 1.25+23.75 µg | TR+SU | 24-32 | - | - | 20-28 |
| VANCOMYCIN | 30 µg | VAN30 | - | - | - | 20-27 |

Q.C. ranges for antimicrobial agents against QC strains for disk diffusion testing are set using data from predefined structured multi-laboratory studies (1). It involves at least 7 independent laboratories, testing on 3 separate lots of medium from 2 different manufactures at least 30 times, using 2 separate disk lots from 2 manufacturers.

REFERENCES


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# EUCAST-and CLSI potency NEO-SENSITABS™

## Interpretation Zones and MIC Breakpoints according to CLSI



Zone diameter interpretative criteria ROSCO and breakpoints when testing *Corynebacteria* are listed in the table below. No zone breakpoints are available from CLSI.

**Table 3.17-1 Interpretation for *Corynebacteria***

**Mueller-Hinton + 5 % blood+20 mg/l β-NAD. Inoculum: McFarland 0.5. Incubation for 24-48 hours at 35 °C ± 2 degrees.**

| NEO-SENSITABS | | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| a) | Cefepime | 30 µg | FEP30 | ≥ 26 | 25-22 | < 22 | ≤ 1 | > 4 |
| a) | Cefotaxime | 30 µg | CTX30 | ≥ 26 | 25-22 | < 22 | ≤ 1 | > 4 |
| a) | Ceftriaxone | 30 µg | CTR30 | ≥ 26 | 25-22 | < 22 | ≤ 1 | > 4 |
| | Ciprofloxacin | 5 µg | CIPR5 | ≥ 26 | 25-19 | ≤ 18 | ≤ 1 | ≥ 4 |
| | Clindamycin | 2 µg | CLI.2 | ≥ 21 | 20-15 | ≤ 14 | ≤ 0.5 | ≥ 4 |
| | Daptomycin | 30 µg | DAPCa | | | | | |
| | 2+18 hours' prediffusion | | | ≥ 22 | - | - | ≤ 1 | - |
| | Doxycycline | 30 µg | DOX30 | ≥ 20 | 19-17 | ≤ 16 | ≤ 4 | ≥ 16 |
| | Erythromycin | 15 µg | ERY15 | ≥ 23 | 22-14 | ≤ 13 | ≤ 0.5 | ≥ 8 |
| | Gentamicin | 10 µg | GEN10 | ≥ 18 | 17-15 | ≤ 14 | ≤ 4 | ≥ 16 |
| a) b) | Imipenem | 10 µg | IMI10 | ≥ 26 | 25-23 | < 22 | ≤ 0.25 | > 1 |
| | Linezolid | 30 µg | LINEZ | ≥ 23 | 22-21 | ≤ 20 | ≤ 2 | ≥ 8 |
| a) b) | Meropenem | 10 µg | MRP10 | ≥ 26 | 25-23 | ≤ 22 | ≤ 0.25 | > 1 |
| a) | Penicillin | 10 U | PEN10 | ≥ 22 | 21-19 | ≤ 18 | ≤ 1 | ≥ 4 |
| | Quinupristin-Dalfopristin | 15 µg | SYN15 | ≥ 19 | 18-16 | ≤ 15 | ≤ 1 | ≥ 4 |
| | Rifampicin | 5 µg | RIF.5 | ≥ 26 | 25-21 | ≤ 20 | ≤ 1 | ≥ 4 |
| | Teicoplanin | 30 µg | TPN30 | ≥ 16 | - | - | ≤ 4 | - |
| | Tetracycline | 30 µg | TET30 | ≥ 24 | 23-19 | ≤ 18 | ≤ 4 | ≥ 16 |
| | Trimethoprim | 1.25 µg | SxT25 | ≥ 16 | 15-11 | ≤ 10 | ≤ 2/38 | ≥ 8/152 |
| | +Sulfa | +23.75 µg | | | | | | |
| | Vancomycin | 30 µg | VAN30 | ≥ 17 | - | - | ≤ 2 | - |

a) Interpretative criteria may not apply to meningitis

b) For detection of possible resistance, ROSCO MIC breakpoints (S ≤ 0.25 ug/ml, R > 1 ug/ml) are recommended for Imipenem and Meropenem. M45-A2 recommends S ≤ 4 ug/ml and R ≥ 16 ug/ml.

MIC breakpoints for carbapenems have been placed at S ≤ 0.25 ug/ml in order to be able to detect isolates with increased resistance to carbapenems.

Testing of isolates from normally sterile sources (blood cultures, deep tissue, prothetic devices) may be warranted, especially in immunodeficient patients.

Cephalosporins MIC interpretative criteria are adopted from those of ***Streptococcus* spp.**, Linezolid adopted from ***Enterococcus* spp.**; remaining from those of ***Staphylococcus* spp.** (CLSI, M45-A2)

Resistant results can be reported after 24 hours. Beta-lactam susceptible results should be reincubated and reported after 48 hours.

Penicillin results are predictive of susceptibility to cephalosporins and carbapenems.

Zone diameters tentative for 1 year.

***For further information see: http://www.eucast.org/eucast_disk_diffusion_test/breakpoints/***

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Zone diameter interpretative standards are correlated to the MIC breakpoints recommended by EUCAST for anaerobic bacteria (1), but zone diameter interpretative standards have not yet been established by EUCAST. The table will be updated with when these are established.

**Table 3.14-1 Interpretation for *Anaerobes***

**Supplemented Brucella Blood agar. Inoculum: McFarland 1.0. Incubation at 35 °C in anaerobic environment for 24-48 hours.**

| NEO-SENSITABS | | | Zone diameter in mm | | | Break-points MIC µg/ml | |
|---|---|---|---|---|---|---|---|
| | | | S | I | R | S | R |
| Amoxycillin+Clav. | 20+10 µg | AMC30 | ≥ 24 | 23-21 | < 21 | ≤ 4 | ≥ 8 |
| Chloramphenicol | 30 µg | CLR30 | ≥ 23 | 22-21 | < 21 | ≤ 8 | > 8 |
| Clindamycin | 2 µg | CLI.2 | ≥ 13 | - | < 13 | ≤ 4 | > 4 |
| Doripenem | 10 µg | DOR10 | ≥ 26 | - | < 26 | ≤ 1 | > 1 |
| Ertapenem | 10 µg | ERTAP | ≥ 26 | - | < 26 | ≤ 1 | > 1 |
| Ertapenem Screen MBL | | | | | < 18 | | possible |
| Imipenem | 10 µg | IMI10 | ≥ 24 | 23-19 | < 19 | ≤ 2 | ≥ 8 |
| Meropenem | 10 µg | MRP10 | ≥ 24 | 23-19 | < 19 | ≤ 2 | ≥ 8 |
| Metronidazole | 16 µg | MTR16 | ≥ 24 | 23-21 | < 21 | ≤ 4 | > 4 |
| Metronidazole | 5 µg | MTR.5 | ≥ 20 | - | < 20 | ≤ 4 | > 4 |
| Penicillin (Ampicillin) | 1 U | PENG1 | ≥ 21 | 20-18 | < 18 | ≤ 0.25 | ≥ 0.5 |
| Piperacillin+Tazobactam | 30+6µg | PIZ36 | ≥ 20 | 19-17 | < 17 | ≤ 8 | ≥ 16 |
| Ticarcillin+Clavulanate | 75+10 µg | TIM85 | ≥ 26 | 25-22 | < 22 | ≤ 8 | ≥ 16 |
| Vancomycin | 5 µg | VAN.5 | ≥ 15 | - | < 15 | ≤ 2 | > 2 |

Zone diameter interpretative standards and MIC breakpoints recommended by EUCAST for Clostridium difficile (1)

**Table 3.14-2 Interpretation for *Clostridium difficile***

**Supplemented Brucella Blood agar. Inoculum: McFarland 1.0. Incubation at 35 °C in anaerobic environment for 24-48 hours.**

| NEO-SENSITABS | | | | Zone diameter in mm | | | Break-points MIC µg/ml | |
|---|---|---|---|---|---|---|---|---|
| | | | | S | I | R | S | R |
| b) | Metronidazole | 16 µg | MTR16 | ≥ 26 | - | < 26 | ≤ 2 | > 2 |
| | Metronidazole | 5 µg | MTR.5 | ≥ 23 | - | < 23 | ≤ 2 | > 2 |
| | Moxifloxacin | 5 µg | MOXIF | ≥ 20 | - | < 20 | ≤ 4 | > 4 |
| | Vancomycin | 5 µg | VAN.5 | ≥ 19 | - | < 19 | ≤ 2 | > 2 |

**References:**


1) EUCAST January 2013.

# EUCAST-and CLSI potency NEO-SENSITABS™

## Interpretation according to MIC Breakpoints of SFM (France) Using CLSI Potency Neo-Sensitabs

Rapidly Growing Bacteria

Rapidly growing bacteria

**Interpretation zones and MIC breakpoints adapted to recommendations by the "Comité de l'Antibiogramme de la Societé Française de Microbiologie" (2012)**

**Inoculum, media and incubation conditions acc. to SFM (2012)**

| | | | | Zone diameter in mm | | | Concentrations critiques | |
|---|---|---|---|---|---|---|---|---|
| | **NEO-SENSITABS** | **POTENCY** | **CODE** | **S** | **I** | **R** | **S** | **R** |
| | Amikacin | 30 µg | AMI30 | ≥ 17 | 16-15 | < 15 | ≤ 8 | > 16 |
| c) | Amoxycillin+Clav. | 20+10 µg | AMC30 | ≥ 21 | 20-16 | < 16 | ≤ 4/2 | > 8/2 |
| a) | Ampicillin | 10 µg | AMP10 | | | | | |
| | Enterobacteriaceae, *E. faecalis* | | | ≥ 19 | 18-16 | < 16 | ≤ 4 | > 8 |
| b) | Ampicillin+Sulbactam | 10+10 µg | SAM20 | ≥ 19 | 18-16 | < 16 | ≤ 4/8 | > 8/8 |
| | Azithromycin | 15 µg | AZI15 | ≥ 22 | 21-17 | < 17 | ≤ 0.5 | > 4 |
| | *Salmonella/Shigella* | | | | | | ≤ 16 | |
| o) g) | Aztreonam | 30 µg | AZT30 | ≥ 27 | 26-21 | < 21 | ≤ 1 | > 8 |
| | Ps. aeruginosa | | | ≥ 27 | 26-19 | < 19 | ≤ 1 | > 16 |
| o) g) | Cefepime | 30 µg# | FEP30 | ≥ 24 | 23-21 | < 21 | ≤ 1 | > 4 |
| | Ps. aeruginosa | | | ≥ 19 | - | < 19 | ≤ 8 | > 8 |
| | Acinetobacter spp. | | | ≥ 21 | 20-19 | < 19 | ≤ 4 | > 8 |
| | Cefepime+Clavulanate | 30+10µg** | CP+CL | | Detection of ESBL | | | |
| g) | Cefixime | 5 µg | CFM.5 | ≥ 22 | 21-19 | < 19 | ≤ 1 | > 2 |
| g) | Cefotaxime | 30 µg | CTX30 | ≥ 26 | 25-23 | < 23 | ≤ 1 | > 2 |
| | Cefotaxime+Clav. | 30+10µg** | CZ+CL | | Detection of ESBL | | | |
| | Cefoxitin | 30 µg | CFO30 | ≥ 22 | 21-15 | < 15 | ≤ 8 | > 32 |
| | *Staphylococcus* spp | | | ≥ 27 | - | < 25 | Oxa S | Mec A pos. |
| | Ceftobiprole | 30 µg | | ≥ 24 | - | - | ≤ 2 | - |
| g) m) | Cefpodoxime (Screen ESBL) | 10 µg | CPD10 | ≥ 24 | 23-21 | < 21 | ≤ 1 | > 2 |
| o) g) | Ceftazidime | 30 µg | CAZ30 | ≥ 26 | 25-21 | < 21 | ≤ 1 | > 4 |
| | Ps. aeruginosa | | | ≥ 19 | - | < 19 | ≤ 8 | > 8 |
| | Acinetobacter spp. | | | ≥ 21 | 20-19 | < 19 | ≤ 4 | > 8 |
| | Ceftazidime+Clav. | 30+10µg** | CZ+CL | | Detection of ESBL | | | |
| g) | Ceftizoxime | 30 µg | ZOX30 | ≥ 26 | 25-23 | < 23 | ≤ 1 | > 2 |
| | Ceftriaxone | 30 µg | CTR30 | ≥ 26 | 25-23 | < 23 | ≤ 1 | > 2 |
| | Cefuroxime (parenteral) | 30 µg | CXM30 | ≥ 22 | - | < 22 | ≤ 8 | > 8 |
| | Cefuroxime (oral) | 30 µg# | CXM30 | ≥ 30 | 29-23 | < 23 | ≤ 1 | > 4 |
| | Cephalothin | 30 µg | CEP30 | ≥ 18 | 17-12 | < 12 | ≤ 8 | > 32 |
| | Chloramphenicol | 30 µg | CLR30 | ≥ 23 | - | < 23 | ≤ 8 | > 8 |
| | Ciprofloxacin | 5 µg | CIPR5 | ≥ 25 | 24-22 | < 22 | ≤ 0.5 | > 1 |
| | *P. aeruginosa* | | | ≥ 25 | 24-22 | < 22 | ≤ 0.5 | > 1 |
| | *Staphylococcus* spp; *Acinetobacter* spp. | | | ≥ 22 | - | < 22 | ≤ 1 | > 1 |
| | Clindamycin | 2 µg# | CLI.2 | ≥ 23 | 22-20 | < 20 | ≤ 0.25 | > 0.5 |
| u) | Cloxacillin | | CLOXA | Detection of plasmid med. Amp C | | | | |
| t) | Colistin | 10 µg*# | CO.10 | | | | | |
| t) x) | 2 + 18 h. pre-diffusion | | | ≥ 15 | 14-11 | ≤ 10 | ≤ 2 | > 2 |
| | Ps. aeruginosa (prediffusion) | | | ≥ 15 | - | < 15 | ≤ 4 | > 4 |
| | Acinetobacter (prediffusion) | | | ≥ 20 | - | - | ≤ 2 | - |
| | Doxycycline | 30 µg# | DOX30 | ≥ 19 | 18-17 | <17 | ≤ 4 | > 8 |
| x) | Daptomycin | 30 µg | DAPCa | | | | | |

# EUCAST-and CLSI potency NEO-SENSITABS™

## Interpretation according to MIC Breakpoints of SFM (France) Using CLSI Potency Neo-Sensitabs

Rapidly Growing Bacteria

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Concentrations critiques S | R |
|---|---|---|---|---|---|---|---|---|
| | 2 + 18 h. prediffusion | | | | | | | |
| t) | *Staphylococcus* spp. | | | ≥ 22 | - | < 20 | ≤ 1 | > 1 |
| | *Enterococcus* spp. | | | ≥ 12 | - | ≤ 11 | ≤ 4 | > 8 |
| | Doripenem | 10 µg | DOR10 | ≥ 24 | 23-19 | < 19 | ≤ 1 | > 4 |
| | Ertapenem | 10 µg | ETP10 | ≥ 28 | 27-26 | < 26 | ≤ 0.5 | > 1 |
| | Erythromycin | 15 µg# | ERY15 | ≥ 22 | 21-19 | < 19 | ≤ 1 | > 2 |
| r) | Fosfomycin | 200 µg# | FO200 | ≥ 18 | - | < 18 | ≤ 32 | > 32 |
| | Fucidin | 10 µg# | FUC10 | ≥ 24 | - | < 24 | ≤ 1 | > 1 |
| | Gatifloxacin | 5 µg | GATIF | ≥ 21 | 20-18 | < 18 | ≤ 1 | ≥ 2 |
| n) | Gentamicin | **10 µg#** | GEN10 | ≥ 18 | 17-16 | < 16 | ≤ 2 | > 4 |
| | *Staphylococcus* spp. | | | ≥ 20 | - | < 20 | ≤ 1 | > 1 |
| | *P. aeruginosa, Acinetobacter* spp. | | | ≥ 16 | - | < 16 | ≤ 4 | >4 |
| h) | Gentamicin (HNR) | **250 µg**** | GN250 | ≥ 14 | - | < 12 | ≤ 250 | > 500 |
| | Imipenem | 10 µg | IMI10 | ≥ 24 | 23-17 | < 17 | ≤ 2 | > 8 |
| | Pseudomonas | | | ≥ 22 | 21-17 | < 17 | ≤ 4 | > 8 |
| | Imipenem+EDTA | 10+750 µg** | IM10E | Detection of metallo-ß-lactamases | | | | |
| q) | Kanamycin | **30 µg#** | KAN30 | ≥ 17 | 16-15 | < 15 | ≤ 8 | > 16 |
| h) | Kanamycin (HNR) | **500 µg**** | KA500 | ≥ 14 | - | < 10 | ≤ 250 | > 500 |
| | Levofloxacin | 5 µg | LEVOF | ≥ 20 | 19-17 | < 17 | ≤ 1 | > 2 |
| | *Acinetobacter* spp; P. aeruginosa | | | ≥ 20 | 19-17 | < 17 | ≤ 1 | > 2 |
| | *Staphylococcus* spp., *Enterococcus* spp. | | | ≥ 20 | 19-17 | < 17 | ≤ 1 | > 2 |
| | Linezolid | 30 µg | LINEZ | ≥ 24 | - | < 24 | ≤ 4 | > 4 |
| | *Staphylococcus* spp., *Enterococcus* spp. | | | ≥ 24 | - | < 24 | ≤ 4 | > 4 |
| | Moxifloxacin | 5 µg | MOXIF | ≥ 24 | 23-21 | < 21 | ≤ 0.5 | > 1 |
| | *Acinetobacter* spp., *Enterococcus* spp. | | | ≥ 21 | 20-18 | < 18 | ≤ 1 | > 2 |
| | Mecillinam (U) | 10 µg | MEC10 | ≥ 24 | 23-22 | < 22 | ≤ 8 | > 8 |
| | Meropenem | 10 µg | MRP10 | ≥ 22 | 21-15 | < 15 | ≤ 2 | > 8 |
| | Minocycline | 30 µg# | MIN30 | ≥ 19 | 18-17 | < 17 | ≤ 4 | > 8 |
| | Staphylococci | | | ≥ 23 | 22-21 | < 21 | ≤ 0.5 | > 1 |
| | Mupirocin (staph) | 10 µg*# | MUPIR | ≥ 19 | 18-10 (LLR) | no zone | ≤ 2 | > 256 (HLR) |
| p) | Nalidixan (U) | 30 µg | NAL30 | ≥ 20 | 19-15 | < 15 | ≤ 8 | > 16 |
| | Enterobacteriaceae | | | - | - | < 19 | Reduced susceptibility to quinolones | |
| | Netilmicin | 30 µg | NET30 | ≥ 21 | 20-19 | < 19 | ≤ 2 | > 4 |
| | *P. aeruginosa, Acinetobacter* spp. | | | ≥ 19 | - | < 19 | ≤ 4 | > 4 |
| | Nitrofurantoin (U) | 300 µg | NI300 | ≥ 15 | - | < 15 | ≤ 64 | > 64 |
| | Norfloxacin (U) | 10 µg# | NORFX | ≥ 25 | 24-22 | < 22 | ≤ 0.5 | > 1 |
| | Ofloxacin | 5 µg | OFL.5 | ≥ 25 | 24-22 | <22 | ≤ 0.5 | > 1 |
| | Oxacillin 1 µg | 1 µg# | OXA.1 | | | | | |
| f) | *Staph. aureus* | | | ≥ 16 | - | < 16 | ≤ 2 | > 2 |
| | Coag. neg. staph. | | | ≥ 20 | - | < 20 | ≤ 0.25 | > 0.5 |

# EUCAST-and CLSI potency NEO-SENSITABS™

## Interpretation according to MIC Breakpoints of SFM (France) Using CLSI Potency Neo-Sensitabs

Rapidly Growing Bacteria

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Concentrations critiques S | R |
|---|---|---|---|---|---|---|---|---|
| | Penicillin | 10 U | PEN10 | | | | | |
| | *Staphylococcus* spp. | | | ≥ 29 | - | < 29 | ≤ 0.12 | beta-lactamase |
| | Piperacillin | 100 µg# | PIPRA | | | | | |
| a) | Enterobacteriaceae | | | ≥ 20 | 19-16 | < 16 | ≤ 8 | > 16 |
| | *P. aeruginosa, Acinetobacter* spp. | | | ≥ 18 | - | < 18 | ≤ 16 | > 16 |
| | Piperacillin+Tazobactam | 100+10µg# | PI+TZ | | | | | |
| e) | Enterobacteriaceae | | | ≥ 21 | 20-17 | < 17 | ≤ 8/4 | > 16/4 |
| e) | *P. aeruginosa, Acinetobacter* spp. | | | ≥ 19 | - | < 19 | ≤ 16/4 | > 16/4 |
| | Quinu/Dalfopristin | 15 µg | SYN15 | ≥ 22 | 21-19 | < 19 | ≤ 1 | > 2 |
| | Enterococcus | | | ≥ 22 | 21-16 | < 16 | ≤ 1 | > 4 |
| | Rifampicin | 5 µg | RIF.5 | | | | | |
| | *Staphylococcus* spp. | | | ≥ 26 | 25-22 | < 22 | ≤ 0.06 | > 0.5 |
| | Ps. aeruginosa;Acinetobacter | | | ≥ 16 | 15-12 | < 12 | ≤ 4 | > 16 |
| | Streptomycin | **10 µg#** | STR10 | ≥ 15 | 14-13 | < 13 | ≤ 8 | > 16 |
| h) | Streptomycin (HNR) | **500 µg**** | ST500 | ≥ 14 | - | < 12 | ≤ 250 | > 500 |
| | Sulphonamides (U) | 240 µg*# | SULFA | ≥ 17 | 16-12 | < 12 | ≤ 64 | > 256 |
| k) s) | Teicoplanin | 30 µg | TPN30 | ≥ 17 | - | - | ≤ 2 | > 8 |
| t) x) | 2 + 18 h. prediffusion (MH agar plain) | *Staphylococcus* spp. | | ≥ 22 | - | < 20 | ≤ 2 | > 8 (VISA/GISA) |
| | Telithromycin | 15 µg | TEL15 | ≥ 21 | 20-17 | < 17 | ≤ 0.5 | > 2 |
| | Tetracyclines | 30 µg# | TET30 | ≥ 19 | 18-17 | < 17 | ≤ 4 | > 8 |
| | Staphylococci | | | ≥ 23 | 22-21 | < 21 | ≤ 1 | > 2 |
| a) | Ticarcillin | 75 µg | TIC75 | ≥ 24 | 23-22 | < 22 | ≤ 8 | > 16 |
| | Pseudomonas | | | ≥ 22 | - | < 22 | ≤ 16 | > 16 |
| d) o) | Ticarcillin+Clavulanate | 75+10 µg | TI+CL | ≥ 24 | 23-22 | < 22 | ≤ 8/2 | > 16/2 |
| | Pseudomonas | | | ≥ 22 | - | < 22 | ≤ 16/2 | > 16/2 |
| | Tigecycline (enterob) | 15 µg | TIG15 | ≥ 21 | 20-19 | < 19 | ≤ 1 | > 2 |
| | Staphylococcus | | | ≥ 22 | - | < 22 | ≤ 0.5 | > 0.5 |
| | Enterococcus | | | ≥ 22 | - | < 22 | ≤ 0.5 | > 0.5 |
| | Acinetobacter | | | ≥ 22 | - | - | ≤ 1 | - |
| | Tobramycin | 10 µg | TOB10 | ≥ 18 | 17-16 | < 16 | ≤ 2 | > 4 |
| | *Staphylococcus* spp. | | | ≥ 20 | - | < 20 | ≤ 1 | > 1 |
| | *P. aeruginosa, Acinetobacter* spp. | | | ≥ 16 | - | < 16 | ≤ 4 | ≥ 4 |
| | Trimethoprim (U) | 5 µg | TRIM5 | ≥ 20 | 19-16 | < 16 | ≤ 2 | > 4 |
| i) | Trimethoprim+Sulfa | 1.25+23.75µg | SxT25 | ≥ 16 | 15-13 | < 13 | ≤ 2/38 | > 4/76 |

| NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Concentrations critiques S | R |
|---|---|---|---|---|---|---|---|
| k) s) Vancomycin | 30 µg | VAN30 | ≥ 17 | - | - | ≤ 2 | > 8 |
| t) x) 2 + 18 h. prediffusion *Staphylococcus* spp.(MH agar plain) | | | ≥ 22 | - | ≤ 20 | ≤ 2 | > 2 (VISA/GISA) |
| *Enterococcus* spp. | | | ≥ 16 | - | < 16 | - | VRE |
| Phenylboronic acid | | BORON | Detection of AmpC and KPC beta lactamases | | | | |
| Dipicolinic acid | | D.P.A | Detection of metallo beta lactamases | | | | |
| ESBL Confirm Kits | | | Confirmation of ESBL's | | | | |
| ESBL+ AmpC Screen Kit | | | Sreening of ESBL and AmpC | | | | |
| Total ESBL + AmpC Confirm Kit | | | Confirmation of ESBL, AmpC and ESBL+AmpC | | | | |
| AmpC Confirm Kit | | | Confirmation of AmpC | | | | |
| KPC, MBL Confirm Kit | | | Confirmation of KPC, MBL | | | | |
| KPC, MBL, OXA-48 Confirm Kit | | | Confirmation of KPC, MBL and OXA-48 | | | | |

Rapidly growing bacteria includes Enterobacteriaceae, *Pseudomonas* spp., *Acinetobacter* spp., *Staphylococcus* spp., *Enterococcus* spp.

#) Differences of potency recommended by SFM and CLSI (if any available).
*) There are no potency recommendations from CLSI so far.
**) Special potency Neo-Sensitabs for detection of resistance mechanisms.

a) *Klebsiella* spp. produces a natural low level beta-lactamase that inactivates amino-, carboxy- and ureido-penicillins. They may appear susceptible in vitro, but they should be reported as Intermediate to carboxy- and ureido-penicillins.

b) Critical concentrations of Ampicillin with a fixed concentration of Sulbactam (8 µg/ml).

c) Critical concentrations of Amoxycillin with a fixed concentration of Clavulanic acid (2 µg/ml).

d) Critical concentrations of Ticarcillin with a fixed concentration of Clavulanic acid (2 µg/ml).

e) Critical concentrations of Piperacillin with a fixed concentration of Tazobactam (4 µg/ml).

f) For detecting Methicillin/Oxacillin resistance in staphylococci follow the instructions enclosed with NeoSensitabs™. Test Cefoxitin Neo-Sensitabs and Oxacillin 1 µg.
Strains resistant to Oxacillin should be reported as resistant to all beta-lactams, even if they appear susceptible in vitro.

g) Strains of *Klebsiella* spp. and *E. coli* may be clinically resistant to cephalosporins and Aztreonam therapy by producing ESBL (extended spectrum beta-lactamase). Read more in "Detection of resistant mechanisms using Neo-Sensitabs™ and Diatabs™.

h) These high content Neo-Sensitabs are used to detect high level resistance to the aminoglycosides.

i) The interpretation of results is valid for other combinations of Trimethoprim+Sulphonamide.

k) Strains showing inhibition zones smaller than the limit for susceptible should be tested by an MIC method.

l) Used for the detection of metallo-ß-lactamases in gram-negatives. See further information in user guide "Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs™".

m) Strains showing zone < 21 mm with Cefpodoxime Neo-Sensitabs, should be suspected of producing ESBL (*E. coli*, Klebsiella, Salmonella).

For ESBL confirmatory tests see user guide "Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs™" ESBL Confirm Kits.

n) Results with Gentamicin and staphylococci are also valid for Netilmicin. Staphylococci that are resistant to Gentamicin, should be reported as resistant against both Netilmicin and Tobramycin (enzymes APH(2")+AAC(6')).

o) Synergism between Ticarcillin+Clavulanate and Aztreonam/Ceftazidime/Cefepime permits the detection of ESBL producing strains in *Ps. aeruginosa*.

p) Nalidixic acid is useful to detect strains with reduced susceptibility to quinolones in 1) Enterobacteriaceae (Nali zone < 19 mm) 2) *Haemophilus influenzae* (Nali zone < 21 mm) 3) Gonococci (Nali zone < 25 mm) and 4) *Vibrio cholerae* (Nali zone < 19 mm).

q) With staphylococci, the interpretation is valid for Amikacin and Isepamicin.

r) Do not take into account the presence of colonies inside the zone of inhibition. Resistant strains show homogeneous resistance.

s) For detection of VISA, GISA, hVISA strains, see user guide "Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs™", on detection of staphylococci with decreased susceptibility to Vancomycin. hVISA, GISA, VRE and Daptomycin Kit.

t) Dipicolinic acid is used for detection of metallo-ß-lactamases. For further information about methodology, see user guide "Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs™". Total MBL Confirm Kit; KPC, MBL Confirm Kit.

u) Cloxacillin and phenylboronic Acid are used for the detection of plasmid-mediated AmpC beta-lactamases. See decription of the procedure in user guide "Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs™" AmpC Confirm Kit.

x) Special technique for susceptibility testing of high molecular weight antimicrobials (Colistin, Daptomycin, Teicoplanin, Vancomycin): 2+18 hours' prediffusion method permitting a good separation between susceptible and resistant strains. Description of the procedure can be found in document **1.5.0**.

Fastidious organisms

**Interpretations zones and MIC breakpoints adapted to recommendations by the "Comité de l'Antibiogramme de la Societé Française de Microbiologie" (2012)**

**Inoculum, media and incubation conditions acc. to SFM (2012)**

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Concentrations critiques S | R |
|---|---|---|---|---|---|---|---|---|
| | Ampicillin | **10 µg** | AMP10 | | | | | |
| e) | *Haemophilus* spp. | | | | Use Ampi 2.5 µg | | - | > 1 |
| | *Campylobacter* spp. | | | ≥ 19 | 18-14 | < 14 | ≤ 4 | > 16 |
| e) | Ampicillin | **2 µg**** | AMP.2 | | | | | |
| | *Haemophilus* spp. | | | ≥ 20 | - | < 20 | ≤ 1 | > 1 |
| | Penicillin | 10 U | PEN10 | | | | | |
| | *N. gonorrhoeae* | | | ≥ 34 | 33-23 | < 23 | ≤ 0.06 | > 1 |
| | Oxacillin | **1 µg#** | OXA.1 | | | | | |
| a) | *S. pneumoniae* | | | ≥ 20 | - | < 20 | ≤ 0.06 (pen) | > 1 MIC test |
| d) | *Streptococcus* spp. | | | ≥ 15 | - | < 15 | ≤ 0.25 (pen) | MIC test |
| l) | *N. gonorrhoeae* | | | ≥ 14 | - | < 14 | ≤ 0.06 (pen) | > 1MIC test |
| g) | *N. meningitidis* | | | ≥ 12 | - | < 12 | ≤ 0.06 (pen) | > 1MIC test |
| | Amoxycillin+Clav. | 20+10 µg | AMC30 | | | | | |
| | *Haemophilus* spp./Moraxella | | | ≥ 24 | - | < 24 | ≤ 1 | > 1 |
| | *Campylobacter* spp. | | | ≥ 21 | 20-14 | < 14 | ≤ 4/2 | > 16/2 |
| | Anaerobes | | | ≥ 21 | 20-17 | < 17 | ≤ 4/2 | > 8/2 |
| | Ticarcillin+Clavulanate | 75+15 µg | TI+CL | | | | | |
| | Anaerobes | | | ≥ 24 | 23-22 | < 22 | ≤ 8/2 | > 16/2 |
| | Piperacillin+Tazobactam | 100+10µg* | PI+TZ | | | | | |
| | Anaerobes | | | ≥ 21 | 20-19 | < 19 | ≤ 8/4 | > 16/4 |
| | Cephalothin | 30 µg | CEP30 | | | | | |
| e) | *Haemophilus* spp. | | | - | - | < 17 | - | > 8 (Amp R) |
| | Campylobacter | | | ≥ 18 | 17-12 | < 12 | ≤ 8 | > 32 |
| | Ceftizoxime | 30 µg** | ZOX30 | | | | | |
| b) | *S. pneumoniae* | | | ≥ 28 | - | < 28 | ≤ 0.5 | > 0.5 |
| | (valid for 3rd gen. cephalosporins) | | | | | | | |
| | Cefotaxime | 30 µg | CTX30 | | | | | |
| | / Ceftriaxone | 30 µg | CTR30 | | | | | |
| b) | *S. pneumoniae* | | | (use Ceftizoxime) | | | ≤ 0.5 | > 2 |
| | H. influenzae | | | ≥ 32 | - | - | ≤ 0.12 | - |
| | *N. gonorrhoeae* | | | ≥ 40 | - | - | ≤ 0.12 | - |
| | *N. meningitidis* | | | ≥ 38 | - | - | ≤ 0.12 | - |
| | *Campylobacter* spp. | | | ≥ 26 | 25-23 | < 23 | ≤ 1 | > 2 |
| | Cefoxitin | 30 µg | CFO30 | | | | | |
| | Anaerobes | | | - | - | < 19 | - | > 32 |
| | Imipenem | 10 µg | IMI10 | | | | | |
| | *S. pneumoniae/streptococci* | | | ≥ 24 | - | < 24 | ≤ 2 | > 2 |
| | *Anaerobes* | | | ≥ 24 | 23-17 | < 17 | ≤ 2 | > 8 |
| | Doripenem | 10 µg | DOR10 | ≥ 23 | - | < 23 | ≤ 1 | > 1 |
| | Meropenem | 10 µg | MRP10 | | | | | |
| | *S. pneumoniae/streptococci* | | | ≥ 24 | - | < 24 | ≤ 2 | > 2 |
| | N. meningitidis | | | ≥ 34 | 33-32 | < 32 | ≤ 0.25 | > 0.25 |
| | Anaerobes | | | ≥ 22 | 21-15 | < 15 | ≤ 2 | > 8 |
| | Ertapenem | 10 µg | ERTAP | | | | | |
| | *S. pneumonia/streptococci* | | | ≥ 28 | 27-24 | < 24 | ≤ 0.5 | - |
| | Anaerobes | | | ≥ 26 | - | < 26 | ≤ 1 | > 1 |
| | Haemophilus/Moraxella | | | ≥ 30 | - | - | ≤ 0.5 | - |
| | Azithromycin | 15 µg | AZI15 | | | | | |

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Concentrations critiques S | R |
|---|---|---|---|---|---|---|---|---|
| | Haemophilus | | | ≥ 28 | 27-14 | < 14 | ≤ 0.12 | > 4 |
| | N.gonorrhoeae | | | ≥ 27 | 26-24 | < 24 | ≤ 0.25 | > 0.5 |
| | Erythromycin | 15 µg# | ERY15 | | | | | |
| c) | *S. pneumoniae* / *Streptococcus* spp. | | | ≥ 26 | 25-24 | < 24 | ≤ 0.25 | > 0.5 |
| | *Campylobacter* spp. | | | ≥ 22 | 21-17 | < 17 | ≤ 1 | > 4 |
| n) | *Helicobacter pylori* | | | ≥ 22 | 21-17 | < 17 | ≤ 1 | > 4 |
| | Clindamycin | 2 µg# | CLI.2 | | | | | |
| | *S. pneumoniae* / *Streptococcus* spp. | | | ≥ 19 | - | < 19 | ≤ 0.5 | > 0.5 |
| | Anaerobes | | | ≥ 15 | - | < 15 | ≤ 4 | > 4 |
| | Linezolid | 30 µg | LINEZ | | | | | |
| | *S. pneumoniae* / *Streptococcus* spp. | | | ≥ 24 | - | < 24 | ≤ 2 | > 4 |
| | Anaerobes | | | ≥ 24 | - | < 24 | ≤ 2 | |
| | Telithromycin | 15 µg | TEL15 | | | | | |
| | *S. pneumoniae* / *Streptococcus* spp. | | | ≥ 24 | 23-21 | < 21 | ≤ 0.25 | > 0.5 |
| | Tetracyclines | 30 µg# | TET30 | | | | | |
| | *Haemophilus* spp. | | | ≥ 23 | 22-20 | < 20 | ≤ 1 | > 2 |
| | *S. pneumoniae* / *Streptococcus* spp. | | | ≥ 23 | 22-21 | < 21 | ≥ 1 | > 2 |
| | *N. gonorrhoeae (tet M)* | | | - | - | < 19 | ≤ 0.5 | > 1 |
| | *Campylobacter* spp. | | | ≥ 19 | 18-17 | < 17 | ≤ 4 | > 8 |
| | Minocycline | 30 µg | MIN30 | | | < 19 | ≤ 0.5 | > 1 |
| | Chloramphenicol | 30 µg | CLR30 | | | | | |
| | *Haemophilus* spp. | | | ≥ 30 | 29-26 | < 26 | ≤ 1 | > 2 |
| | *S. pneumoniae* / *Streptococcus* spp. | | | ≥ 23 | - | < 23 | ≤ 8 | > 8 |
| | *N. gonorrhoeae* | | | ≥ 28 | 27-24 | < 24 | ≤ 4 | > 16 |
| | *N. meningitidis* | | | ≥ 30 | - | - | ≤ 2 | > 4 |
| | *Campylobacter* spp. | | | ≥ 23 | 22-19 | < 19 | ≤ 8 | > 16 |
| | Anaerobes | | | ≥ 23 | - | < 23 | ≤ 8 | > 8 |
| | Rifampicin | 5 µg | RIF.5 | | | | | |
| | *Haemophilus* spp. | | | ≥ 18 | - | < 18 | ≤ 0.5 | > 0.5 |
| | *Streptococcus* spp;Pneumococci | | | ≥ 26 | 25-22 | < 22 | ≤ 0.06 | > 0.5 |
| h) | *N. meningitidis* | | | ≥ 24 | - | - | ≤ 0.25 | - |
| | Anaerobes | | | ≥ 14 | 13-10 | < 10 | ≤ 4 | > 16 |
| | Gentamicin | 10 µg | GEN10 | | | | | |
| | *Campylobacter* spp. | | | ≥ 18 | 17-16 | < 16 | ≤ 2 | > 4 |
| | Tobramycin | 10 µg | TOB10 | | | | | |
| | *Campylobacter* spp. | | | ≥ 18 | 17-16 | < 16 | ≤ 2 | > 4 |
| $c_2$) | Norfloxacin | 10 µg# | NORFX | | | | Reduced susceptibility | |
| | *S. pneumoniae* | | | - | - | < 12 | to quinolones | |
| | Ciprofloxacin | 5 µg | CIPR5 | | | | | |
| | *Haemophilus* spp. | | | use Nalidixic acid | | | ≤ 0.5 | - |
| | *N. gonorrhoeae* | | | use Nalidixic acid | | | ≤ 0.03 | > 0.06 |
| | *Campylobacter* spp. | | | ≥ 25 | 24-22 | < 22 | ≤ 0.5 | > 1 |
| | *Helicobacter pylori* | | | ≥ 20 | - | < 20 | ≤ 1 | > 1 |
| | *S. pneumoniae* | | | ≥ 30 | 29-19 | < 19 | ≤ 0.12 | > 2 |
| | *N. meningitidis* | | | ≥ 36 | 35-32 | < 32 | ≤ 0.03 | > 0.06 |
| | Ofloxacin | 5 µg | OFL.5 | | | | | |
| | *Haemophilus* spp. | | | use Nalidixic acid | | | ≤ 0.5 | - |
| | *N. gonorrhoeae or* | | | use Nalidixic acid | | | ≤ 0.12 | > 0.25 |
| | Levofloxacin | 5 µg | LEVOF | | | | | |
| | *Haemophilus* spp. | | | use Nalidixic acid | | | ≤ 1 | - |
| $c_2$) | *S. pneumoniae* | | | ≥ 17 | - | < 17 | ≤ 2 | > 2 |
| | *Streptococcus* spp. | | | ≥ 20 | 19-17 | < 17 | ≤ 1 | > 2 |
| | Gatifloxacin | 5 µg | GATIF | | | | | |
| | *Haemophilus* spp. | | | use Nalidixic acid | | | ≤ 1 | - |
| $c_2$) | *S. pneumoniae* / *Streptococcus* spp. | | | ≥ 20 | 19-17 | < 17 | ≤ 1 | > 2 |

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Concentrations critiques S | R |
|---|---|---|---|---|---|---|---|---|
| | Moxifloxacin | 5 µg | MOXIF | | | | | |
| | *Haemophilus* spp. | | | use Nalidixic acid | | | ≤ 0.5 | - |
| $c_2$) | *S. pneumoniae* | | | ≥ 24 | - | < 24 | ≤ 0.5 | > 0.5 |
| | *Streptococcus* spp. | | | ≥ 24 | 23-21 | < 21 | ≤ 0.5 | > 1 |
| | Anaerobes | | | ≥ 21 | 20-18 | < 18 | ≤ 1 | > 2 |
| | Nalidixan | 30 µg | NAL30 | | | | | |
| f) | *Haemophilus* spp. | | | - | - | < 21 | decreased susceptibility | |
| k) | *N. gonorrhoeae* | | | - | - | < 25 | to quinolones | |
| i) | *N. meningitidis* | | | - | - | < 21 | - | - |
| | *Campylobacter* spp. | | | ≥ 20 | 19-15 | < 15 | ≤ 8 | > 16 |
| | Trimethoprim+Sulfa | 1.25+23.75µg | SxT25 | | | | | |
| | *Haemophilus* spp. | | | ≥ 24 | - | - | ≤ 0.5/9.5 | > 1/19 |
| | *S. pneumoniae* / *Streptococcus* spp. | | | ≥ 19 | 18-16 | < 16 | ≥ 1/19 | > 2/38 |
| | Gentamicin | 10 µg# | GEN10 | | | | | |
| | *Haemophilus* spp. | | | ≥ 16 | 15-14 | < 14 | ≤ 2 | > 4 |
| | *Campylobacter* spp. | | | ≥ 16 | 15-14 | < 14 | ≤ 2 | > 4 |
| | Tobramycin | 10 µg | TOB10 | | | | | |
| | *Campylobacter* spp. | | | ≥ 18 | 17-16 | < 16 | ≤ 2 | > 4 |
| | Spectinomycin | 200 µg | SPECT | | | | | |
| | N.gonorrhoea | | | ≥ 20 | - | < 20 | ≤ 64 | > 64 |
| | Teicoplanin | 30 µg | TPN30 | | | | | |
| | *S. pneumoniae*/*Streptococcus* spp. | | | ≥ 17 | - | - | ≤ 4 | > 8 |
| | Anaerobes | | | ≥ 17 | - | - | ≤ 4 | > 8 |
| | Tigecycline | | | | | | | |
| | Streptococcus spp. | | | ≥ 22 | - | < 22 | ≤ 0.25 | > 0.5 |
| | Anaerobes | | | ≥ 21 | - | - | ≤ 4 | > 8 |
| | Vancomycin | 30 µg | VAN30 | | | | | |
| | *S. pneumoniae* / *Streptococcus* spp. | | | ≥ 17 | - | - | ≤ 4 | > 8 |
| | Anaerobes | | | ≥ 17 | - | - | ≤ 4 | > 8 |
| m) | Metronidazole | 16 µg* | MTR16 | | | | | |
| | Anaerobes | | | ≥ 21 | - | < 21 | ≤ 4 | > 4 |
| | *Clostridium difficile* | | | ≥ 26 | - | < 26 | ≤ 4 | > 4 |

#) Differences of potency recommended by SFM and CLSI (if any available).
*) There are no potency recommendations from CLSI so far.
**) Special potency Neo-Sensitabs for detection of resistance mechanism.

**Pneumococci**

a) Oxacillin 1 µg is used for the detection of reduced sensitivity to Penicillin in pneumococci. Penicillin resistant isolates from the meninges must be considered resistant to Ampicillin/Amoxycillin, Amox+Clav and first and second generation cephalosporins.

b) Cefotaxime and Ceftriaxone must not be tested against pneumococci by the diffusion method. A surrogate test is used instead: Ceftizoxime, Ceftizoxime detects reduced sensitivity to third generation cephalosporins. Strains sensitive to Ceftizoxime show currently MIC < 0.5 µg/ml towards Cefotaxime/Ceftriaxone (susceptible), while isolates resistant to Ceftizoxime should be tested by an MIC method.

c) Erythromycin: Interpretation valid for Azithromycin and Clarithromycin.

$c_2$) Screening of pneumococci for reduced sensitivity to fluoroquinolones is done using Norfloxacin 10 µg Neo-Sensitabs. If the inhibition zone is < 12 mm (or the MIC is >16 µg/ml) there is a high risk of development of resistant mutants in vivo.

**Streptococci**

Penicillin resistant strains of Group A streptococci have not yet been recognized. Viridans streptococci isolated from blood or CSF should be tested for Penicillin or Ampicillin susceptiblity using an MIC method. Group B streptococci with reduced susceptibility to Penicillin have been isolated.

d) Oxacillin 1 µg is useful for screening for Penicillin susceptibility in viridans streptococci.
c) Erythromycin: Interpretation valid for Azithromycin and Clarithromycin.

***H. influenzae***

e) Beta-lactamase negative, Ampicillin resistant strains (BLNAR) are best detected using Ampicillin 2 µg Neo-Sensitabs. Cephalothin Neo-Sensitabs is also useful to detect BLNAR strains (zone < 17 mm). BLNAR isolates must be considered resistant to Amoxycillin, Amox+Clav, as well as first and second generation cephalosporins, no matter the size of the inhibition zone.
f) Strains resistant to Nalidixic acid should be suspected of having reduced susceptibility to quinolones. Strains with reduced susceptibility to ciprofloxacin (MIC ≥ 0.125 µg/ml) show decreased susceptibility to all quinolones.

**Meningococci**

g) Oxacillin 1 µg is used routinely for the detection of reduced sensitivity to penicillins, in meningococci (chromosomal resistance).
h) Rifampicin: Used for prophylaxis only (not treatment).
i) Nalidixan is useful to screen for strains with reduced susceptibility to quinolones.

**Gonococci**

k) Nalidixan is useful to detect strains with reduced susceptibility to quinolones. Ciprafloxacin resistant gonococci should presumably be resistant to all quinolones.
A positive beta-lactamase test predicts resistance to Penicillin, Amoxycillin/Ampicillin, Piperacillin and Ticarcillin.
l) Oxacillin 1 µg Neo-Sensitabs is useful to detect beta lactamase negative gonococci with decreased susceptibility to Penicillin (chromosomal resistance).

**Campylobacter**

For *Campylobacter* spp. the absence of zone of inhibition around ß-lactams, aminoglycosides, macrolides or quinolones indicates high level resistance.

**Anaerobes**

Vancomycin 5 µg, Kanamycin 500 µg and Colistin 10 µg Neo-Sensitabs are very useful for the identification of the most important gram negative bacilli: *B. fragilis* group are resistant to Vancomycin 5 µg, Kanamycin 500 µg and Colistin 10 µg. Prevotella is resistant to Kanamycin 500 µg and Vancomycin 5 µg (zone < 18 mm), while it is variable to Colistin 10 µg. Porphyromonas is sensitive to Vancomycin 5 µg (zone > 18 mm) and resistant to Kanamycin 500 µg and Colistin 10µg. Fusobacterium is sensitive to Kanamycin 500 µg and Colistin 10 µg and resistant to Vancomycin 5 µg.
For species showing slow growth it may be difficult to establish a correlation between MIC's and zone sizes. Use an MIC method.
m) Metronidazole: Certain strains may show false resistance to Metronidazole if anaerobiosis is not correct.

***Helicobacter pylori***

n) Interpretation valid for Clarithromycin.

**References:**

1) Barbut F. et al: Antimicrobial susceptibilities and serogroups of clinical strains of *Clostridium difficile* isolated in France in 1991 and 1997. Antimicr. Ag. Chemother., **43**, 2607-11,1999.
2) Communique 2004 de la Societe Francaise de Microbiologie (CA-SFM).

3) Communique Janvier 2005 de la Societe Francaise de Microbiologie (CA-SFM).
4) Communique Janvier 2006 de la Societe Francaise de Microbiologie (CA-SFM).
5) Communique Janvier 2007 de la Societe Francaise de Microbiologie (CA-SFM).
6) Communique Janvier 2008 de la Societe Francaise de Microbiologie (CA-SFM).
7) Communique Janvier 2009 de la Societe Francaise de Microbiologie (CA-SFM).
8) Communique Janvier 2010 de la Societe Francaise de Microbiologie (CA-SFM).
9) Communique Janvier 2011 de la Societe Francaise de Microbiologie (CA-SFM).
10) Communique Janvier 2012 de la Societe Francaise de Microbiologie (CA-SFM).

Rapidly growing bacteria

**Interpretation according to the MIC break-points adapted to recommendations in the BSAC Standardized Disc Testing Method (Version 11), UK and Ireland (2012)**

**Media: Iso Sensitest, Inoculum: Semiconfluent growth (ICS) according to BSAC**

| | | | | Zone diameter in mm | | | Break-points MIC µg/ml | |
|---|---|---|---|---|---|---|---|---|
| | **NEO-SENSITABS** | **POTENCY** | **CODE** | **S** | **I** | **R** | **S** | **R** |
| | **Beta-lactams:** | | | | | | | |
| | Penicillin | 1 unit | PENG1 | ≥ 25 | - | ≤ 24 | | |
| a) | Staph. | | | | | | ≤ 0.12 | penase |
| b) | Oxacillin | 1 µg | OXA.1 | | | | | |
| | *Staph. aureus* (MH Agar) | | | ≥ 15 | - | ≤ 14 | ≤ 2 | > 2 |
| | Coag. neg. staph. (MH Agar) | | | ≥ 20 | - | ≤ 19 | ≤ 0.25 | > 0.25 |
| | Ampicillin | 10 µg | AMP10 | ≥ 15 | - | ≤ 14 | ≤ 8 | > 8 |
| | Enterococci | | | ≥ 20 | - | ≤ 19 | ≤ 4 | > 8 |
| | Amoxycillin | 25 µg | AMX25 | ≥ 22 | - | ≤ 21 | ≤ 8 | > 8 |
| | Amoxycillin+Clav. | 20+10 µg | AMC30 | ≥ 21 | - | ≤ 20 | ≤ 8 | > 8 |
| | Mecillinam (U) | 10 µg | MEC10 | ≥ 14 | - | ≤ 13 | ≤ 8 | > 8 |
| | Temocillin | 30 µg | TEMOC | ≥ 20 | - | ≤ 19 | ≤ 8 | > 8 |
| | Urine | | | ≥ 15 | - | ≤ 14 | ≤ 32 | > 32 |
| d) | Piperacillin | 100 µg | PIPER | ≥ 24 | - | ≤ 23 | ≤ 8 | > 16 |
| | Piperacillin+Tazo. | 75+10µg | PTZ85 | ≥ 23 | 22-21 | ≤ 20 | ≤ 16 | > 16 |
| | Pseudomonas spp. | | | ≥ 25 | - | ≤ 24 | <16 | > 16 |
| | Ticarcillin | 75 µg | TIC75 | ≥ 21 | - | ≤ 20 | ≤ 16 | > 16 |
| | *Pseudomonas* spp. | | | ≥ 20 | - | ≤ 19 | ≤ 16 | > 16 |
| k) | Ticarcillin+Clav. | 75+10 µg | TIM85 | ≥ 23 | - | ≤ 22 | ≤ 8 | > 8 |
| | *Pseudomonas* spp. | | | ≥ 20 | - | ≤ 19 | ≤ 16 | > 16 |
| o) | Cloxacillin | | CLOXA | Detection of plasmid-mediated AmpC | | | | |
| | Cephalothin | 30 µg | CEP30 | ≥ 27 | - | ≤ 26 | ≤ 8 | > 8 |
| | Cephalexin | 30 µg | CFLEX | | | | | |
| | Urine (*E. coli*) | | | ≥ 16 | - | ≤ 15 | ≤ 16 | > 16 |
| | Cefuroxime (oral) UTI | 30 µg | CXM30 | ≥ 20 | - | ≤ 19 | ≤ 8 | > 8 |
| | Cefuroxime (inj) | 30 µg | CXM30 | ≥ 20 | - | ≤ 19 | ≤ 8 | > 8 |
| | Cefoxitin | **10 µg** | CFO10 | | | | | |
| | *S. aureus* (Iso-Sensitest) | | | ≥ 22 | - | < 22 | Oxa S | Mec A pos. |
| | *S. aureus* (Mueller-Hinton) | | | ≥ 18 | - | < 18 | Oxa S | Mec A pos. |
| | coag. neg. staph. (Iso-s) | | | ≥ 27 | - | ≤ 21 | Oxa S | Mec A pos. |
| | Cefoxitin | 30 µg | CFO30 | ≥ 23 | - | - | AmpC Screen | |
| d) j) | Cefpodoxime | 10 µg | CPD10 | ≥ 20 | - | ≤ 19 | ≤ 1 | > 1 |
| | (Screen ESBL) | | | - | - | < 24 | | |
| | Cefpodoxime+Clav. | 10+1 µg | CPD+C | Screening for ESBL | | | Screen ESBL | |
| g) d) | Cefotaxime | 30 µg | CTX30 | ≥ 30 | 29-24 | ≤ 23 | ≤ 1 | > 2 |
| | Cefotaxime+Clav. | 30+10 µg | CTX+C | Detection of ESBL | | | | |
| g) d) | Ceftriaxone | 30 µg | CTR30 | ≥ 28 | 29-24 | < 23 | ≤ 1 | > 2 |

# EUCAST-and CLSI potency NEO-SENSITABS™

## Interpretation according to MIC Breakpoints of BSAC (UK and Ireland) using BSAC and CLSI potency Neo-Sensitabs

Rapidly growing bacteria

| NEO-SENSITABS | | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| k) g) d) | Ceftazidime | 30 µg | CAZ30 | ≥ 27 | 26-23 | ≤ 22 | ≤ 1 | > 4 |
| | *Pseudomonas* spp. | | | ≥ 24 | - | ≤ 23 | ≤ 8 | > 8 |
| | Ceftazidime | 10 µg | CAZ10 | ≥ 24 | 23-16 | ≤ 15 | ≤ 1 | > 8 |
| | P. aeruginosa | | | ≥ 20 | - | ≤ 19 | ≤ 8 | > 8 |
| | Acinetobacter | | | ≥ 16 | - | ≤ 15 | ≤ 8 | > 8 |
| | Ceftazidime+Clav. | 30+10µg | CZ+CL | Detection of ESBL | | | | |
| k) d) | Cefepime | 30 µg | FEP30 | ≥ 32 | 31-26 | < 26 | ≤ 1 | > 4 |
| | *Pseudomonas* spp. | | | ≥ 23 | - | ≤ 22 | ≤ 8 | > 8 |
| | Cefepime+Clav. | 30+10µg | CP+CL | Detection of ESBL | | | | |
| k) g) d) | Aztreonam | 30 µg | AZT30 | ≥ 28 | 27-22 | < 22 | ≤ 1 | > 8 |
| | *Pseudomonas* spp. | | | ≥ 36 | 35-20 | ≤ 19 | ≤ 1 | > 16 |
| h) l) | Imipenem | 10 µg | IMI10 | ≥ 21 | 20-17 | ≤ 16 | ≤ 2 | > 8 |
| | *Ent. faecalis* | | | ≥ 19 | 18-17 | ≤ 16 | ≤ 4 | > 8 |
| | *Pseudomonas* spp. | | | ≥ 25 | 24-17 | ≤ 16 | ≤ 4 | > 8 |
| | *Acinetobacter* spp. | | | ≥ 25 | 24-14 | ≤ 13 | ≤ 2 | > 8 |
| p) | Imipenem+EDTA | 10+750µg | IM10E | Detection of metallo-ß-lactamases | | | | |
| h) l) | Meropenem | 10 µg | MRP10 | ≥ 27 | 26-19 | < 19 | ≤ 2 | > 8 |
| h) | *Pseudomonas* spp. | | | ≥ 20 | 19-16 | ≤ 15 | ≤ 2 | > 8 |
| | *Acinetobacter* spp. | | | ≥ 25 | 24-19 | ≤ 18 | ≤ 2 | > 8 |
| | Ertapenem | 10 µg | ETP10 | ≥ 28 | 27-16 | ≤ 15 | ≤ 0.5 | > 1 |
| | Doripenem | 10 µg | DOR10 | ≥ 24 | 23-19 | ≤ 18 | ≤ 1 | > 4 |
| | *Pseudomonas* spp. | | | ≥ 32 | 31-25 | ≤ 24 | ≤ 1 | > 4 |
| | *Acinetobacter* spp. | | | ≥ 24 | 23-18 | ≤ 17 | ≤ 1 | > 4 |
| | *Enterococci* spp. | | | ≥ 22 | 21-19 | ≤ 18 | ≤ 1 | > 4 |
| **Aminoglycosides:** | | | | | | | | |
| | Amikacin | 30 µg | AMI30 | ≥ 19 | 18-16 | ≤ 15 | ≤ 8 | > 16 |
| | *Pseudomonas/Acinetobacter* spp | | | ≥ 22 | 21-16 | ≤ 15 | ≤ 8 | > 16 |
| | Coag. neg. staph. | | | ≥ 25 | 24-22 | ≤ 21 | ≤ 1 | > 1 |
| f) | Gentamicin | **10 µg** | GEN10 | ≥ 20 | 19-16 | < 16 | ≤ 2 | > 4 |
| | *Pseudomonas* | | | > 18 | - | ≤ 17 | ≤ 4 | > 4 |
| | S.aureus; *Acinetobacter* spp. | | | ≥ 20 | - | ≤ 19 | ≤ 1 | > 1 |
| | Coagulase neg. staph. | | | ≥ 28 | - | ≤ 27 | ≤ 0.25 | > 0.25 |
| | Gentamicin (HLR) | **250 µg** | GN250 | - | - | < 17 | - | HLR |
| | Streptomycin | 10 µg(enterob) | STR10 | ≥ 13 | - | < 12 | ≤ 8 | > 8 |
| f) | Tobramycin | 10 µg | TOB10 | ≥ 21 | 20-18 | ≤ 17 | ≤ 2 | > 4 |
| | *Pseudomonas/Acinetobacter* spp. | | | ≥ 20 | - | ≤ 19 | ≤ 4 | > 4 |
| | Staph. aureus | | | ≥ 21 | - | ≤ 20 | ≤ 1 | > 1 |
| | Coagulase neg. staph. | | | ≥ 30 | - | ≤ 29 | ≤ 0.25 | > 0.25 |
| **Other:** | | | | | | | | |
| | Erythromycin | 15 µg | ERY15 | ≥ 20 | 19-17 | ≤ 16 | ≤ 1 | > 2 |
| | Azithromycin | 15 µg | AZI15 | ≥ 20 | - | ≤ 19 | ≤ 1 | > 2 |
| | *Salmonella typhi* | | | ≥ 19 | - | ≤ 18 | - | - |
| | Clarithromycin | 15 µg | CLA15 | ≥ 20 | 19-17 | ≤ 16 | ≤ 1 | > 2 |
| | Clindamycin | 2 µg | CLI.2 | ≥ 26 | 25-23 | ≤ 22 | ≤ 0.25 | > 0.5 |
| | Quinupristin /Dalfopristin | 15 µg | SYN15 | ≥ 22 | 21-19 | <19 | ≤ 2 | > 2 |
| | Enterococcus (E. faecium) | | | ≥ 20 | 19-12 | ≤11 | ≤ 1 | > 4 |
| | Staphylococci | | | ≥ 22 | 21-19 | ≤18 | ≤ 1 | > 2 |

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| | Linezolid | 10 µg | LIZ10 | ≥ 20 | - | ≤ 19 | ≤ 4 | > 4 |
| | Telithromycin | 15 µg | TEL15 | ≥ 27 | - | ≤ 26 | ≤ 0.5 | > 0.5 |
| | Fucidin | 10 µg | FUC10 | ≥ 30 | - | ≤ 29 | ≤ 1 | > 1 |
| | Doxycycline | 30 µg | DOX30 | ≥ 29 | - | ≤ 28 | ≤ 1 | > 1 |
| | Minocycline | 30 µg | MIN30 | | | | | |
| | *Staphylococcus* spp. | | | ≥ 28 | - | ≤ 27 | ≤ 0.5 | > 0.5 |
| | Tetracyclines | 30 µg | TET30 | ≥ 29 | - | ≤ 28 | ≤ 1 | > 1 |
| | Chloramphenicol | 30 µg | CLR30 | ≥ 21 | - | ≤ 20 | ≤ 8 | > 8 |
| | Rifampicin (staph) | 5 µg | RIF.5 | ≥ 32 | 31-26 | ≤ 25 | ≤ 0.06 | > 0.5 |
| n) | Teicoplanin | 30 µg | TPN30 | | | | | |
| q) | 2+18 h prediffusion ( MH agar,McF.0.5) | | | | | | | |
| | *Staphylococcus* spp. | | | ≥ 22 | - | < 20 | ≤ 2 | > 2 (VISA/ GISA) |
| | *Enterococcus* spp. | | | ≥ 20 | - | ≤ 19 | ≤ 2 | > 2 (VRE) - - |
| c) n) | Vancomycin | 30 µg | VAN30 | | | | | |
| q) | 2+18 h prediffusion (MH agar, McF 0.5) | | | | | | | |
| | *Staphylococcus* spp. | | | ≥ 24 | - | ≤ 22 | ≤ 2 | >2 (VISA/GISA) |
| | *Enterococcus* spp. | | | ≥ 16 | 15-12 | < 12 | ≤ 4 | VRE |
| q) | Daptomycin | 30 µg | DAPCa | | | | | |
| | 2+18 hours' prediffusion | | | | | | | |
| | *Staphylococcus* spp. | | | ≥ 22 | 21-20 | < 20 | ≤ 1 | > 2 |
| | *Enterococcus* spp. | | | ≥ 12 | - | ≤ 11 | ≤ 4 | > 8 |
| i) | Nitrofurantoin (U) | 200 µg | NI200 | ≥ 17 | - | ≤ 16 | ≤ 64 | > 64 |
| e) | Nalidixic acid (U) | 30 µg | NAL30 | ≥ 18 | - | ≤ 17 | ≤ 16 | > 16 |
| | *Enterobacteriaceae/Acinetobacter* spp. | | | ≥ 20 | - | ≤ 19 | Reduced susceptibility to quinolones | |
| | *Salmonella* spp. | | | ≥ 20 | - | ≤ 19 | Reduced susceptibility to quinolones | |
| | Norfloxacin (syst.) | 2 µg | NOR2 | ≥ 26 | 25-19 | ≤ 18 | ≤ 0.5 | > 1 |
| | Norfloxacin (UTI) | 2 µg | NOR2 | ≥ 16 | - | ≤ 15 | ≤ 4 | > 4 |
| e) | Ciprofloxacin | 1 µg | CIPR1 | ≥ 20 | 19-17 | ≤ 16 | ≤ 0.5 | > 1 |
| | *Staphylococcus* spp. | | | ≥ 14 | - | ≤ 13 | ≤ 1 Red. suscept. quinolones | > 1 |
| | *Pseudomonas* spp. | | | ≥ 21 | - | ≤ 20 | ≤ 1 | > 1 |
| | Urine | | | ≥ 20 | - | ≤ 19 | ≤ 4 | > 4 |
| | *Acinetobacter* spp. | | | ≥ 21 | - | ≤ 20 | ≤ 1 | > 1 |
| | Ciprofloxacin | 5 µg | CIPR5 | | | | | |
| | *Pseudomonas* spp. | | | ≥ 30 | 29-20 | ≤ 19 | ≤ 0.5 | > 1 |
| e) | Ofloxacin | 5 µg | OFL.5 | ≥ 29 | 28-25 | < 25 | ≤ 0.5 | > 1 |
| e) | Levofloxacin | 5 µg | LEVOF | ≥ 22 | 21-19 | ≤ 18 | ≤ 2 | > 2 |
| | *Pseudomonas* | | | ≥ 22 | 21-17 | ≤ 16 | ≤ 1 | > 2 |
| q) | Colistin | 10 µg | Co.10 | | | | | |
| q) | 2+18 hours' prediffusion | | | ≥ 15 | 14-11 | ≤ 10 | ≤ 2 | > 2 |
| | Gatifloxacin | 5 µg | GATIF | ≥ 28 | 27-24 | ≤ 23 | ≤ 0.5 | > 1 |
| e) | Moxifloxacin | 5 µg | MOXIF | ≥ 28 | 27-24 | ≤ 23 | ≤ 0.5 | > 1 |

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| | *Pseudomonas* | | | ≥ 31 | 30-25 | ≤ 24 | ≤ 0.5 | > 1 |
| | *Staph.* | | | ≥ 20 | 19-16 | ≤ 15 | ≤ 0.5 | > 1 |
| | Sulphonamides (U) | 240 µg | SULFA | ≥ 28 | - | ≤ 27 | ≤ 32 | > 32 |
| | Trimethoprim | 2.5 µg | TP2.5 | ≥ 20 | 19-15 | ≤ 14 | ≤ 0.5 | > 4 |
| | Enterococci (U) | | | ≥ 33 | 32-26 | ≤ 25 | ≤ 0.03 | > 1 |
| | *Staph.* | | | ≥ 20 | - | ≤19 | ≤ 1 | > 1 |
| | Urine | | | ≥ 17 | 16-14 | ≤ 13 | ≤ 2 | > 4 |
| m) | Trimethoprim + Sulfa | 1.25+23.75µg | SxT25 | ≥ 16 | - | ≤ 15 | ≤ 2 | > 4 |
| | Staphylococcci | | | ≥ 17 | 16-14 | ≤ 13 | ≤ 2 | > 4 |
| | *S. maltophilia* | | | ≥ 20 | - | ≤ 19 | ≤ 4 | > 4 |
| | Fosfomycin (U) | 200+50 µg | FO200 | ≥ 25 | - | ≤ 24 | ≤ 32 | > 32 |
| | *Staph.* | | | ≥ 34 | - | ≤ 33 | ≤ 32 | > 32 |
| | Novobiocin (U) | 5 µg | NOVO5 | ≥ 16 | - | ≤ 15 | - | - |
| | Mupirocin (staph) | 10 µg | MUPIR | ≥ 18 | 17-10 (LLR) | ≤ 9 (HLR) | ≤ 4 | > 128 |
| | Tigecycline | 15 µg | TIG15 | | | | | |
| r) | *Enterobacteriaceae/Acinetobacter* spp. | | | ≥ 24 | 23-20 | ≤ 19 | ≤ 1 | > 2 |
| | *Staphylococcus* spp. | | | ≥ 26 | - | ≤ 25 | ≤ 0.5 | > 0.5 |
| | *Enterococcus* spp. | | | ≥ 21 | - | ≤ 20 | ≤ 0.25 | > 0.5 |
| | **Special tests** | | | | | | | |
| o) | Boronic Acid | | BORON | Detection of AmpC and KPC beta lactamases | | | | |
| p) | Dipicolinic Acid | | D.P.A. | Detection of metallo-ß-lactamases | | | | |

Rapidly growing bacteria includes Enterobacteriaceae., *Pseudomonas* spp., *Acinetobacter* spp., *Staphylococcus* spp., *Enterococcus* spp.

**Remarks:**

a) Staphylococci resistant to Penicillin should be reported as resistant to Amoxycillin, Ampicillin, Piperacillin and Ticarcillin.

b) Iso-sensitest will not reliably detect resistance of staphylococci towards Oxacillin (test Cefoxitin). Use Mueller-Hinton Agar (BSAC 2001). Staphylococci resistant to Cefoxitin (Oxacillin) should be reported resistant to **all** other beta-lactams including beta-lactamase inhibitior combinations and carbapenems. Cefoxitin testing on Iso-sensitest agar should be performed at a temperature not exceeding 35°C to avoid false susceptibility results (Skov et al. (7))

c) When testing enterococci against Vancomycin 5 µg, plates should be incubated for full 24 hours and examined carefully for the presence of a haze or other growth within the zone (indicates resistance).

d) Strains of Klebsiella, *E. coli* and Salmonella that produce ESBL may be clinically resistant to therapy with Penicillin, cephalosporins or Aztreonam, despite apparent in vitro susceptibility. Read in Neo-Sensitabs User's Guide 20th Ed. 2009 on ESBL screening and confirmatory tests.

e) Enterobacteriaceae resistant to Nalidixic acid (zone < 20 mm) show a **decreased** susceptibility to quinolones MIC CIPRO ≥ 0.125 µg/ml). Therefore Nalidixic acid is a good screening for the detection of decreased quinolone susceptibility in *Salmonella* spp. Strains of *E. coli*, Klebsiella and Salmonella with Ciprofloxacin MIC's of 0.25 and 0.5 µg/ml may be reported as resistant to the quinolones. According to Wareham et al. (4) *E. coli* and *K. pneumonia* isolates with plasmid-mediated quinolone resistance had a zone diameter of ≤ 16mm around Ciprofloxacin 1 µg disks.

f) Staphylococci that are **resistant** to Gentamicin should be reported as resistant against both Netilmicin and Tobramycin (enzymes APH (2") + AAC (6°)). Staphylococci resistant to Kanamycin should be reported as resistant to Amikacin.

h) According to BSAC zones for Meropenem and P. aeruginosa should be S ≥ 20 mm, I: 19-16, R ≤ 15 mm. Several carbapenemase producing isolates will be falsely reported as susceptible to Meropenem.

i) Klebsiella, Enterobacter and *Proteus* spp. should be reported **R** to Nitrofurantoin.

j) *E. coli*/Klebsiella/Salmonella strains showing zone < 24 mm with Cefpodoxime Neo-Sensitabs should be suspected of producing ESBL. For confirmatory tests use Ceftazidime+Clavulanate and Cefepime+Clavulanate compared to Ceftazidime and Cefepime Neo-Sensitabs (see ESBL).

k) Synergism between Ticarcillin+Clavulanate and Aztreonam/Ceftazidime/Cefepime permit the detection of ESBL producing strains (*Ps. aeruginosa*).

l) Carbapenem testing on Iso-sensitest Agar may give falsely susceptibility for isolates that harbour metallo-beta-lactamases, testing on Mueller-Hinton Agar is to be preferred (BSAC 2001) (2)

m) For *S. maltophilia and* B. cepacia use incubation at 30 °C. (3,6) For B. cepacia complex should be developed specific criteria.

n) For detection of VISA, GISA, hVISA strains, see chapter in Neo-Sensitabs User's Guide 20th Edition 2009 on detection of staphylococci with decreased susceptibility to Vancomycin.

o) Cloxacillin and Phenylboronic acid are used for the detection of plasmid-mediated AmpC beta-lactamases. See description of the procedure in Neo-Sensitabs User's Guide 20th Ed. 2009. For further information on methodology, see leaflet "Screening and detection of AmpC beta lactamases" (www.rosco.dk).

p) Imipenem+EDTA and Dipicolinic acid are used for the detection of metallo-ß-lactamases. See further information in User's Guide "Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs™ 2010". For further information on methodology, see leaflet "Screening and detection of carbapenemases" (www.rosco.dk).

q) Special technique for susceptibility testing of high molecular weight antimicrobials (Colistin, Daptomycin, Teicoplanin, Vancomycin): 2+18 hours' prediffusion method. Description can be found in document **1.5.0**.

r) Disk duffusion valid only for E. coli. For Acinetobacter use MIC method. According to Hope et al. (5) the best advice for Tigecycline testing is to perform broth dilution tests on isolates with borderline results, especially in severe infections and when Tigecycline use is intended.

**References:**

1) BSAC Standardized Disc Testing Method (last update Version 11) (2012).

2) Wootton M. et al: Examinig the effect of media on carbapenem resistance interpretation in P. aeruginosa. Abstract presentation D-730, 49th ICAAC, 2009.
3) Pitman K. et al: Comparison of methods for suceptibility testing of clinically important Burkholderia spp. Abstract presentation D-1443, 49th ICAAC, 2009.
4) Amin A., Wareham D.: Ability of Ciprofloxacin 1 µg Discs and BSAC Breakpoints to Detect Plasmid-Mediated Quinolone Resistance in Urinary Enterobacterial isolates. Abstract P586, 20th ECCMID, Vienna, April 2010.
5) Hope R. et al: Tigecycline activity: low resistance rates but problematic disc breakpoints revealed by a multicentre sentinel survey in the UK. J. Antimicr. Chemother. 65, 2602-2609, 2010.
6) Pitman K et al: Disc susceptibility testing for Burkholderia cepacia complex. Presentation D-752, 50th ICAAC, Sept. 2010.
7) Skov R et al: Effects of temperature on the detection of methicillin resistance in S. aureus using Cefoxitin disc diffusion testing with Iso-sensitest agar. J. Antimicr. Chemother. **63**, 699-703, 2009.

Fastidious organisms

**Interpretation according to the MIC break-points adapted to recommendations by the BSAC (Version 11) in UK and Ireland.**

**Inoculum, media and incubation conditions according to BSAC (2012).**

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| | Ampicillin | **10 µg** | AMP10 | | | | | |
| e) | *Haemophilus* spp. | | | | Use Ampi 2.5 µg | | ≤ 1 | > 1 |
| | *Moraxella catarrhalis* | | | ≥ 30 | - | ≤ 29 | ≤ 1 | beta lact.pos |
| | *Pasteurella multocida* | | | ≥ 30 | - | ≤ 29 | ≤ 1 | > 1 |
| e) | Ampicillin | **2 µg** | AMP.2 | | | | | |
| | *Haemophilus* spp. | | | ≥ 18 | - | ≤ 17 | ≤ 1 | > 1 |
| | Streptococci | | | ≥ 24 | 23-15 | ≤ 14 | ≤ 0.5 | > 2 |
| | Moraxella catarrhalis | | | ≥ 30 | - | ≤ 29 | ≤ 1 | > 1 |
| | *Pasteurella multocida* | | | ≥ 20 | - | ≤ 19 | ≤ 1 | > 1 |
| | *N. meningitidis* | | | ≥ 32 | - | ≤ 31 | - | - |
| | Penicillin | 1 unit | PENG1 | | | | | |
| a) | Streptococci haemol. | | | ≥ 20 | - | ≤ 19 | ≤ 0.25 | > 0.25 |
| | α-haem strep. | | | ≥ 17 | 16-11 | ≤ 10 | ≤ 0.25 | > 2 |
| | *N. gonorrhoeae* | | | ≥ 26 | 25-18 | ≤ 17 | ≤ 0.06 | > 0.12 |
| | *N. meningitidis* | | | ≥ 29 | 28-15 | ≤ 14 | ≤ 0.06 | > 0.25 |
| | Coryneforms | | | ≥ 20 | - | ≤ 19 | ≥ 0.12 | > 0.12 |
| | *Moraxella catarrhalis* | | | BL neg. | - | BL pos. | S | R |
| | *Pasteurella multocida* | | | ≥ 22 | - | ≤ 21 | ≤ 0.12 | beta lact.pos |
| | Anaerobes ( Clostridium) | | | ≥ 23 | - | ≤ 22 | ≤ 0.25 | > 0.5 |
| | Oxacillin | 1 µg | OXA.1 | | | | | |
| a) | *S. pneumoniae* | | | ≥ 20 | 19-11 | ≤ 10 | ≤ 0.06 (pen) | 2(MICtest) |
| d) | *Streptococcus* spp. | | | ≥ 17 | - | ≤ 16 | ≤ 0.12 (pen) | MIC test |
| l) | *N. gonorrhoeae* | | | ≥ 15 | - | ≤ 14 | ≤ 0.06 (pen) | MIC test |
| g) | *N. meningitidis* | | | ≥ 12 | - | ≤ 11 | ≤ 0.06 (pen) | MIC test |
| | Amoxycillin+Clav. | 2+1 µg | AMC.3 | | | | | |
| | *Haemophilus* spp. | | | ≥ 14 | - | ≤ 13 | ≤ 2 | > 2 |
| | *Moraxella catarrhalis* | | | ≥ 19 | - | ≤ 19 | ≤ 1 | > 1 |
| | Amoxycillin+Clav. | 20+10 µg | AMC30 | | | | | |
| | *Haemophilus* spp. | | | ≥ 29 | - | ≤ 28 | ≤ 1 | > 1 |
| | *Moraxella catarrhalis* | | | ≥ 29 | - | ≤ 28 | ≤ 1 | > 1 |
| | Anaerobes | | | ≥ 29 | 28-21 | ≤ 20 | ≤ 4 | > 8 |
| | *Clostridium* | | | ≥ 32 | - | ≤ 31 | ≤ 4 | > 8 |
| | Piperacillin+Tazobactam | 75+10µg | PTZ85 | | | | | |
| | Anaerobes | | | ≥ 27 | - | ≤ 26 | ≤ 16 | > 16 |
| | Cefaclor | 30 µg | CCL30 | | | | | |
| | *Haemophilus* spp. | | | ≥ 28 | - | ≤ 27 | ≤ 0.5 | > 0.5 |
| | *Moraxella catarrhalis* | | | ≥ 38 | - | ≤ 37 | ≤ 0.12 | > 0.12 |
| | Cefuroxime | 30 µg | CXM30 | | | | | |

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| | *Haemophilus* spp. | | | ≥ 32 | - | ≤ 31 | ≤ 1 | > 2 |
| | *N. gonorrhoeae* | | | ≥ 38 | - | ≤ 37 | ≤ 1 | > 1 |
| | *Moraxella catarrhalis* | | | ≥ 24 | - | ≤ 23 | ≤ 4 | > 8 |
| | Cefixime | 5 µg | CFM.5 | ≥ 35 | - | ≤ 34 | ≤ 0.12 | > 0.12 |
| | N.gonorrhoae | | | | | | | |
| | Cefotaxime | 5 µg | CTX.5 | | | | | |
| | *Haemophilus* spp. | | | ≥ 25 | - | ≤ 24 | ≤ 0.12 | > 0.12 |
| | *Pasteurella multocida* | | | ≥ 34 | - | ≤ 33 | ≥ 1 | > 1 |
| | *N. meningitidis* | | | ≥ 40 | - | ≤ 39 | ≤ 0.12 | > 0.12 |
| | *N. gonorrhoeae* | | | ≥ 30 | - | ≤ 29 | ≤ 0.12 | > 0.12 |
| | *Streptococcus* spp. | | | ≥ 23 | | ≤ 22 | ≤ 0.5 | > 0.5 |
| b) | *S. pneumoniae* | | | | use ceftizoxime | | - | - |
| | Ceftriaxone | 30 µg | CTR30 | | | | | |
| | *Haemophilus* spp. | | | ≥ 38 | - | ≤ 37 | ≤ 0.12 | > 0.12 |
| b) | *S. pneumoniae* | | | | use ceftizoxime | | - | - |
| | *N. gonorrhoeae* | | | ≥ 38 | - | ≤ 37 | ≤ 0.12 | > 0.12 |
| | *N. meningitidis* | | | ≥ 38 | - | ≤ 37 | ≤ 0.12 | > 0.12 |
| | *Streptococcus* spp. | | | ≥ 35 | - | ≤ 34 | ≤ 0.5 | > 0.5 |
| | Ceftizoxime | 30 µg | ZOX30 | | | | | |
| b) | *S. pneumoniae* | | | ≥ 30 | 29-24 | ≤ 23 | ≤ 1 | MIC |
| | (valid for 3rd gen. cepha.) | | | | | | | |
| | Imipenem | 10 µg | IMI10 | | | | | |
| | *S. pneumoniae* | | | ≥ 25 | - | ≤ 24 | ≤ 2 | > 2 |
| | *Haemophilus* spp. | | | ≥ 23 | - | ≤ 22 | ≤ 2 | > 2 |
| | Meropenem | 10 µg | MRP10 | | | | | |
| | *S. pneumoniae* | | | ≥ 28 | - | ≤ 27 | ≤ 2 | > 2 |
| | *S. pneumoniae (meningitidis)* | | | ≥ 32 | 31-28 | <28 | ≤ 0.25 | > 1 |
| | *Haemophilus* spp. | | | ≥ 23 | - | ≤ 22 | ≤ 2 | > 2 |
| | *Haemophilus* spp.( meningitidis) | | | ≥ 34 | 33-30 | <30 | ≤ 0.25 | > 1 |
| | Anaerobes * | | | ≥ 26 | 25-19 | ≤ 18 | ≤ 2 | > 8 |
| | Ertapenem | 10 µg | ETP10 | | | | | |
| | *S. pneumoniae* | | | ≥ 33 | - | ≤ 32 | ≤ 0.5 | > 0.5 |
| | Streptococci | | | ≥ 35 | - | ≤ 34 | ≤ 0.5 | > 0.5 |
| | *M. catarrhalis* | | | ≥ 35 | - | ≤ 34 | ≤ 0.5 | > 0.5 |
| | *Haemophilus* spp. | | | ≥ 33 | - | ≤ 32 | ≤ 0.5 | > 0.5 |
| | Doripenem | 10 µg | DOR10 | | | | | |
| | Streptococci | | | | | | | |
| | *S. pneumoniae* | | | ≥ 23 | - | ≤ 22 | ≤ 1 | > 1 |
| | Erythromycin | 15 µg | ERY15 | | | | | |
| c) | *S. pneumoniae/Streptococcus* spp. | | | ≥ 24 | 23-22 | ≤ 21 | ≤ 0.25 | > 0.5 |
| | Moraxella cat | | | ≥ 28 | - | ≤ 27 | ≤ 0.25 | > 0.5 |
| | Campylobacter | | | ≥ 22 | - | ≤ 21 | ≤ 4 | > 4 |
| | Haemophilus spp. | | | ≥ 32 | 31-16 | ≤ 15 | ≤ 0.25 | > 32 |
| | Azithromycin | 15 µg | AZI15 | | | | | |
| | *Haemophilus* spp. | | | ≥ 35 | 34-20 | ≤ 19 | ≤ 0.12 | > 4 |
| m) | *N. gonorrhoeae* | | | ≥ 28 | - | ≤ 27 | ≤ 0.25 | > 0.5 |
| | Streptococci, *S. pneumoniae* | | | ≥ 22 | 21-20 | ≤ 19 | ≤ 0.25 | > 0.5 |
| | Clarithromycin | 15 µg | CLA15 | | | | | |
| | *Moraxella catarrhalis* | | | ≥ 28 | 27-26 | ≤ 25 | ≤ 0.25 | > 0.5 |
| | Streptococci, *S. pneumoniae* | | | ≥ 24 | 23-22 | ≤ 21 | ≤ 0.25 | > 0.5 |
| | *Campylobacter* spp. | | | ≥ 20 | - | ≤ 19 | ≤ 1 | > 1 |

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| | *Haemophilus* spp. | | | ≥ 24 | 23-10 | no zone | ≤ 1 | > 32 |
| | Clindamycin | 2 µg | CLI.2 | | | | | |
| | *S. pneumoniae*/streptococci | | | ≥ 20 | - | ≤ 19 | ≤ 0.5 | > 0.5 |
| | Anaerobes | | | ≥ 10 | - | no zone | ≤ 4 | > 4 |
| | Quinupristin /Dalfopristin | 15 µg | SYN15 | | | | | |
| | *S. pneumoniae* | | | ≥ 20 | - | ≤ 19 | ≤ 2 | > 2 |
| | Linezolid | 10 µg | LIZ10 | | | | | |
| | *Moraxella catarrhalis* | | | ≥ 20 | - | ≤ 19 | ≤ 2 | > 4 |
| | *S. pneumoniae*/*Streptococcus* spp. | | | ≥ 20 | - | ≤ 19 | ≤ 4 | > 4 |
| | Telithromycin | 15 µg | TEL15 | | | | | |
| | *S. pneumoniae* | | | ≥ 29 | - | ≤ 28 | ≤ 0.25 | > 0.5 |
| | *Streptococcus* spp. | | | ≥ 29 | - | ≤ 28 | ≤ 0.25 | > 0.5 |
| | *M. catarrhalis* | | | ≥ 30 | - | ≤ 29 | ≤ 0.25 | > 0.5 |
| | *Haemophilus* spp. | | | ≥ 31 | 30-16 | ≤ 15 | ≤ 0.12 | > 8 |
| | Tetracyclines | 30 µg | TET30 | | | | | |
| | *S. pneumoniae*/*Streptococcus* spp. | | | ≥ 26 | - | ≤ 25 | ≤ 1 | > 2 |
| | *Campylobacter* spp. | | | ≥ 22 | 21-19 | ≤ 18 | ≤ 2 | > 2 |
| | *N. gonorrhoeae* | | | ≥ 36 | 35-30 | ≤ 29 | ≤ 0.5 | > 1 |
| | *P. multocida* | | | ≥ 30 | - | ≤ 29 | ≤ 1 | > 1 |
| | *Haemophilus* | | | ≥ 26 | 25-22 | ≤ 21 | ≤ 1 | > 2 |
| | Tigecycline | 15 µg | TIG15 | | | | | |
| | *Streptococcus* spp. | | | ≥ 25 | 24-20 | ≤ 19 | ≤ 0.25 | > 0.5 |
| | *Haemophilus* spp. | | | ≥ 28 | - | ≤ 27 | ≤ 0.25 | > 0.25 |
| | Chloramphenicol | 30 µg | CLR30 | | | | | |
| | *S. pneumoniae* | | | ≥ 21 | - | ≤ 20 | ≤ 8 | > 8 |
| | *N. meningitidis* | | | ≥ 26 | - | ≤ 25 | ≤ 2 | > 4 |
| | Haemophilus spp. | | | ≥ 30 | 29-27 | ≤ 26 | ≤ 2 | > 2 |
| | *Moraxella catarrhalis* | | | ≥ 32 | - | ≤ 31 | ≤ 1 | > 2 |
| | Rifampicin | 5 µg | RIF.5 | | | | | |
| | *S. pneumoniae* | | | ≥ 23 | 22-21 | ≤ 20 | ≤ 0.06 | > 0.5 |
| h) | *N. meningitidis* | | | ≥ 34 | - | ≤ 33 | ≤ 0.25 | > 0.25 |
| | Vancomycin | 30 µg | VAN30 | | | | | |
| | *S. pneumoniae*/streptococci | | | ≥ 18 | - | ≤ 17 | ≤ 4 | > 4 |
| | Coryneforms | | | ≥ 20 | - | ≤ 19 | ≤ 4 | > 8 |
| | *Cl. difficile* | | | - | - | - | ≤ 2 | > 2 |
| | Nalidixic acid (U) | 30 µg | NAL30 | | | | | |
| f) | *Haemophilus* spp. | | | ≥ 25 | - | no zone | decreased | |
| | *Pasteurella multocida* | | | ≥ 28 | - | ≤ 27 | susceptibility to | |
| k) | *N. gonorrhoeae* | | | ≥ 32 | 31-10 | no zone | quinolones | |
| | *Moraxella catarrhalis (screen)* | | | ≥ 18 | - | ≤ 17 | | |
| | *Campylobacter* spp. | | | ≥ 20 | - | ≤ 19 | | |
| | Norfloxacin | 2 µg | NOR2 | | | | decreased susceptibility | |
| | *S. pneumoniae* | | | - | - | < 10 | to quinolones | |

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | Zone diameter in mm I | Zone diameter in mm R | Break-points MIC µg/ml S | Break-points MIC µg/ml R |
|---|---|---|---|---|---|---|---|---|
| | Ciprofloxacin | 1 µg | CIPR1 | | | | | |
| f) | *Haemophilus* spp. | | | ≥ 28 | - | ≤ 27 | ≤ 0.5 | > 0.5 |
| | *Campylobacter* spp. | | | ≥ 26 | - | ≤ 25 | ≤ 1 | > 1 |
| | *Pasteurella multocida* | | | ≥ 29 | - | ≤ 28 | ≤ 1 | > 1 |
| | *S. pneumoniae* | | | ≥ 25 | 24-10 | ≤ 9 | ≤ 0.12 | > 2 |
| | Coryneforms | | | ≥ 17 | 16-12 | ≤ 11 | ≤ 0.5 | > 1 |
| | *N. meningitidis* | | | ≥ 32 | - | ≤ 31 | ≤ 0.03 | > 0.06 |
| | *N. gonorrhoeae* | | | ≥ 29 | - | ≤ 28 | ≤ 0.03 | > 0.06 |
| | *Moraxella catarrhalis* | | | ≥ 18 | - | ≤ 17 | ≤ 0.5 | > 0.5 |
| | Ofloxacin | 5 µg | OFL.5 | | | | | |
| | *S. pneumoniae* | | | ≥ 28 | 27-16 | ≤ 15 | ≤ 0.12 | > 4 |
| | *M. catarrhalis/Haemophilus* spp. | | | ≥ 35 | - | ≤ 34 | ≤ 0.5 | > 0.5 |
| | Levofloxacin | 5 µg | LEVOF | | | | | |
| | *S. pneumoniae/Streptococcus* spp. | | | ≥ 18 | - | ≤ 17 | ≤ 2 | > 2 |
| | *M. catarrhalis/ Haemophilus* spp. | | | ≥ 28 | - | ≤ 27 | ≤ 1 | > 1 |
| | Moxifloxacin | 5 µg | MOXIF | | | | | |
| | *S. pneumoniae/Streptococcus* spp. | | | ≥ 22 | - | ≤ 21 | ≤ 0.5 | > 0.5 |
| | *Moraxella catarrhalis* | | | ≥ 30 | - | ≤ 29 | ≤ 0.5 | > 0.5 |
| | *Haemophilus* spp. | | | ≥ 30 | - | ≤ 29 | ≤ 0.5 | > 0.5 |
| | *Cl. difficile* | | | - | - | - | - | > 4 |
| | Nitrofurantoin | 200 µg | NI200 | | | | | |
| | Streptococci | | | ≥ 19 | - | ≤ 18 | ≤ 64 | > 64 |
| | Trimethoprim | 2.5 µg | TP2.5 | | | | | |
| | Haemophilus spp. | | | ≥ 21 | - | ≤ 20 | ≤ 0.5 | > 0.5 |
| | Trimethoprim+ Sulfa | 1.25+23.75µg | SxT25 | | | | | |
| | *Haemophilus* spp. | | | ≥ 21 | 20-18 | ≤ 17 | ≤ 0.5 | > 1 |
| | Streptococci, *S. pneumoniae* | | | ≥ 20 | 19-17 | ≤ 16 | ≤ 1 | > 2 |
| | *Moraxella catarrhalis* | | | ≥ 17 | - | ≤ 16 | ≤ 0.5 | > 1 |
| | Spectinomycin | 200 µg | SPECT | | | | | |
| | *N. gonorrhoeae* | | | ≥ 23 | 22-20 | ≤ 19 | ≤ 64 | > 64 |
| | Metronidazole | 5 µg | MTR.5 | | | | | |
| | Anaerobes | | | ≥ 18 | - | ≤ 17 | ≤ 4 | > 4 |
| | *Cl. difficile* | | | - | - | - | ≤ 2 | > 2 |
| m) | Metronidazole | 16 µg | MTR16 | | | | | |
| | *Campylobacter* spp. | | | ≥ 28 | - | ≤ 27 | ≤ 4 | > 4 |
| | Anaerobes | | | ≥ 24 | - | ≤ 23 | ≤ 4 | > 4 |

**B. fragilis* showing inhibition zones < 30 mm show increased expression of cfiA and should be reported as resistant to meropenem (1).

**Remarks:**

**Pneumococci**

a) Oxacillin 1 µg is used for the detection of reduced sensitivity to Penicillin in pneumococci. Penicillin resistant isolates from the meninges must be considered resistant to Ampicillin/Amoxycillin, Amox+Clav and first and second generation cephalosporins.

b) Cefotaxime and Ceftriaxone must not be tested against pneumococci by the diffusion method. A surrogate test is used instead: Ceftizoxime. Ceftizoxime detects reduced sensitivity to third generation cephalosporins. Strains sensitive to Ceftizoxime show currently MIC < 0.5 µg/ml towards Cefotaxime/Ceftriaxone (susceptible), while isolates resistant to Ceftizoxime should be tested by an MIC method.
c) Erythromycin: Interpretation valid for Azithromycin and Clarithromycin.

**Streptococci**
Penicillin resistant strains of Group A streptococci have not yet been recognized.
Viridans streptococci isolated from blood or CSF should be tested for Penicillin or Ampicillin susceptiblity using an MIC method. Group B streptococci with reduced susceptibility to Penicillin have been isolated.
d) Oxacillin 1 µg is useful for screening for Penicillin susceptibility in streptococci.
c) Erythromycin: Interpretation valid for Azithromycin and Clarithromycin.

***H. influenzae***
e) Beta-lactamase negative, ampicillin resistant strains (BLNAR) are best detected using Ampicillin 2.5 µg Neo-Sensitabs. BLNAR isolates must be considered resistant to Amoxycillin, Amox+Clav, as well as first and second generation cephalosporins, no matter the size of the inhibition zone.
f) Strains resistant to nalidixic acid should be suspected of having reduced susceptibility to quinolones. Strains with reduced susceptibility to Ciprofloxacin (MIC ≥ 0.125 µg/ml) show decreased susceptibility to all quinolones.

**Meningococci**
g) Oxacillin 1 µg is used routinely for the screening of reduced sensitivity to penicillins, in meningococci (chromosomal resistance).
h) Rifampicin: Used for prophylaxis only (not treatment).

**Gonococci**
k) Nalidixic acid is useful to detect strains with reduced susceptibility to quinolones. Ciprafloxacin resistant gonococci should presumably be resistant to all quinolones.
A positive beta-lactamase test predicts resistance to Penicillin, Amoxycillin/Ampicillin, Piperacillin and Ticarcillin.
l) Oxacillin (1 µg) Neo-Sensitabs are useful to detect beta-lactamase negative gonococci with decreased susceptibility to Penicillin (chromosomal resistance).
m) Patients treated for Chlamydia with Azithromycin cannot be assumed to have been adequately treated for gonococcal infection. UK Department of Health recommends that Azithromycin should not be used to treat gonorrhoea.

**Campylobacter**
For *Campylobacter* spp. the absence of zone of inhibition around ß-lactams, aminoglycosides, macrolides or quinolones indicates high level resistance.

**Anaerobes**
Interpretation is valid for Bacteroides spp and Clostridium difficile.
For species showing slow growth it may be difficult to establish a correlation between MIC's and zone sizes. Use an MIC method.
m) Metronidazole: Certain strains may show false resistance to Metronidazole if anaerobiosis is not correct.

**References:**
1) Ang Lei et al: Carbapenem resistance in Bacteroides fragilis. J.A.C., **59**, 1043-44, 2007.

# EUCAST-and CLSI potency NEO-SENSITABS™

## Interpretation according to MIC breakpoints of BSAC (UK and Ireland) using BSAC potency Neo-Sensitabs

BSAC urines

BSAC urines

**MIC and zone diameter breakpoints for isolates from URINES, according to BSAC 2012**

**Neo-Sensitabs potency according to BSAC. Iso-sensitest Agar. Inoculum according to BSAC.**

| NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|
| Amoxycillin | 25 µg | AMX25 | | | | | |
| Enterobacteriaceae | | | ≥ 22 | - | ≤ 21 | ≤ 8 | > 8 |
| Enterococci | | | ≥ 20 | - | ≤ 19 | | |
| S. saprophyticus | | | ≥ 26 | - | ≤ 25 | | |
| Group B streptococci | | | ≥ 26 | - | ≤ 25 | | |
| Amoxycillin+Clav. | 20+10 µg | AMC30 | | | | | |
| Enterobacteriaceae | | | ≥ 13 | - | ≤ 12 | ≤ 32 | > 32 |
| Enterococci | | | ≥ 21 | - | ≤20 | | |
| S. saprophyticus | | | ≥ 28 | - | ≤27 | | |
| Group B streptococci | | | ≥ 28 | - | ≤27 | | |
| Ampicillin | 10 µg | AMP10 | | | | | |
| Enterobacteriaceae | | | ≥ 15 | - | ≤ 14 | ≤ 8 | > 8 |
| Cefepime | 30 µg | FEP30 | | | | | |
| Enterobacteriaceae | | | ≥ 32 | 31-26 | < 26 | ≤ 1 | > 8 |
| Pseudomonas | | | ≥ 23 | - | ≤ 22 | ≤ 8 | > 8 |
| Cefepime+Clav. | 30+10 µg | FEP+C | | Detection ESBL | | | |
| Cefotaxime | 30 µg | CTX30 | ≥ 30 | 29-24 | ≤ 23 | ≤ 1 | > 2 |
| Cefotaxime+Clav | 30+10 µg | CTX+C | | Detection ESBL | | | |
| Cefpodoxime | 30 µg | CPD10 | ≥ 20 | - | ≤ 19 | ≤ 1 | Screen ESBL |
| Cefpodoxime+Clav. | 10+1 µg | CPD+C | | Screening ESBL | | | |
| Ceftazidime | 30 µg | CAZ30 | | | | | |
| Enterobacteriaceae | | | ≥ 30 | 29-17 | <17 | ≤ 1 | > 8 |
| Pseudomonas | | | ≥ 26 | - | ≤ 25 | ≤ 8 | > 8 |
| Cefatzidime+Clav. | 30+10 µg | CAZ+C | | Detection EBSL | | | |
| Cefuroxime | 30 µg | CXM30 | ≥ 20 | - | ≤ 19 | ≤ 8 | > 8 |
| Cephalexin | 30 µg | CFLEX | | | | | |
| E.coli/Klebsiella | | | ≥ 16 | - | ≤ 15 | ≤ 32 | > 32 |
| P. mirabilis | | | ≥ 12 | - | ≤ 11 | | |
| Group B streptococci | | | ≥ 24 | - | ≤ 23 | | |
| Ciprofloxacin | 1 µg | CIPR1 | | | | | |
| Enterobacteriaceae | | | ≥ 20 | 19-17 | ≤ 16 | ≤ 0.5 | > 1 |
| Enterococci | | | ≥ 12 | - | ≤ 11 | | |
| S. saprophyticus | | | ≥ 18 | - | ≤ 17 | | |
| Group B streptococci | | | ≥ 13 | - | ≤ 12 | | |
| Ertapenem | 10 µg | ETP10 | ≥ 28 | - | ≤ 27 | ≤ 0.5 | > 1 |
| Fosfomycin | 200+50 µg | FO200 | | | | | |
| E.coli | | | ≥ 25 | - | ≤24 | ≤ 32 | > 32 |
| P. mirabilis | | | ≥ 37 | - | ≤ 36 | | |
| Enterococci | | | ≥ 20 | - | ≤19 | | |
| S. saprophyticus | | | ≥ 20 | - | ≤ 19 | | |
| Gentamicin | 10 µg | GEN10 | ≥ 20 | 19-17 | ≤ 16 | ≤ 2 | > 4 |
| Imipenem | 10 µg | IMI10 | ≥ 21 | 20-17 | ≤16 | ≤ 2 | > 8 |
| Mecillinam | **10 µg** | MEC10 | | | | | |
| E.coli/Klebsiella/P.mirabilis | | | ≥ 14 | - | ≤ 13 | ≤ 8 | > 8 |
| Mecillinam | **33 µg** | MECIL | | | | | |
| S. saprophyticus | | | ≥ 10 | - | no zone | ≤ 64 | > 64 |
| Meropenem | 10 µg | MRP10 | ≥ 27 | 26-20 | ≤ 19 | ≤ 2 | > 8 |
| Nalidixic acid | 30 µg | NAL30 | | | | | |
| Enterobacteriaceae | | | ≥ 18 | - | ≤17 | ≤ 16 | > 16 |
| Nitrofurantoin | 200 µg | NI200 | | | | | |
| E.coli | | | ≥ 17 | - | ≤ 16 | ≤ 64 | > 64 |

# EUCAST-and CLSI potency NEO-SENSITABS™

## Interpretation according to MIC breakpoints of BSAC (UK and Ireland) using BSAC potency Neo-Sensitabs

**BSAC urines**

| NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|
| Enterococci | | | ≥ 20 | - | ≤19 | | |
| S. saprophyticus | | | ≥ 20 | - | ≤19 | | |
| Group B streptococci | | | ≥ 19 | - | ≤18 | ≤ 64 | > 64 |
| Norfloxacin | 2 µg | NOR.2 | ≥ 16 | - | ≤15 | ≤ 4 | > 4 |
| Piperacillin+Tazo | (75+10) µg | TIM85 | ≥ 22 | - | < 21 | ≤ 16 | > 16 |
| Temocillin | 30 µg | TEMOC | | | | | |
| Enterobacteriaceae | | | ≥ 12 | - | ≤11 | ≤ 32 | > 32 |
| Tetracyclines | 30 µg | TET30 | ≥ 29 | - | ≤28 | ≤ 1 | > 1 |
| Tigecycline | 15 µg | TIG15 | ≥ 24 | 23-20 | ≤19 | ≤ 1 | > 2 |
| Trimethoprim | 2.5 µg | TP2.5 | | | | | |
| Enterobacteriaceae | | | ≥ 17 | 16-14 | ≤ 13 | ≤ 2 | ≥ 4 |
| Enterococci | | | ≥ 50 | 49-22 | ≤ 21 | ≤ 0.03 | > 1 |
| S. saprophyticus | | | ≥ 15 | 14-13 | ≤ 12 | ≤ 2 | ≥ 4 |
| Group B streptococci | | | ≥ 16 | - | ≤ 15 | ≤ 2 | ≥ 4 |
| Trimethoprim+Sulfa | 1.25+23.75 µg | SxT25 | ≥ 16 | - | < 15 | ≤ 2 | > 2 |

**Special tests**

| | | | |
|---|---|---|---|
| a) | Cloxacillin | CLOXA | Detection of plasmid mediated AmpC |
| a) | Boronic acid | BORON | Detection of AmpC and KPC beta lactamases |
| b) | Dipicolinic acid | D.P.A | Detection of metallo-ß-lactamases |
| b) | Imipenem+EDTA 10+750 µg | IM10E | Detection of metallo-ß-lactamases |

a) Description of the methodology can be found in a leaflet "Screening and detection of AmpC beta lactamases" and user's guide "Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs™ 2010" (www.rosco.dk).

b) Description of the methodology can be found in a leaflet "Screening and detection of carbapenemases" It also inlcudes KPC and metallo-β-lactamases (www.rosco.dk).

**Interpretation of the Antibiogramme with Neo-Sensitabs**
**MIC break-points according to CLSI (M31-A4/M31-S2 2013)**
**Inoculum according to Kirby-Bauer / confluent colonies.**

**Mueller-Hinton agar and McFarland 0.5 inoculum.**

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| | Amoxicillin+Clav. | 20+10 µg | AMC30 | ≥ 30 | 29-28 | ≤ 27 | ≤ 0.25 | ≥ 1 (dogs, cats) |
| | *Staphylococcus* spp. | | | | Use cefoxitin | | ≤ 4/2 | ≥ 8/4 |
| | Other organisms | (UTI only) | | ≥ 18 | 17-14 | ≤ 13 | ≤ 8/4 | ≥ 32/16 |
| | Amikacin | 30 µg | AMI30 | ≥ 17 | 16-15 | ≤ 14 | ≤ 16 | ≥ 32 |
| | Amoxycillin | 30 µg | AMOXY | | | | | |
| | *Enterococcus* spp. | | | ≥ 20 | - | ≤ 19 | ≤ 8 | ≥ 16 |
| | Other organisms | | | ≥ 20 | 19-17 | ≤ 16 | ≤ 8 | ≥ 32 |
| | Ampicillin | 10 µg | AMP10 | | | | | |
| | Enterobacteriaceae | | | ≥ 17 | 16-14 | ≤ 13 | ≤ 8 | ≥ 32 |
| | *Staphylococcus* spp. | | | | Use penicillin | | | |
| | *Enterococcus* spp. | | | ≥ 17 | - | ≤ 16 | ≤ 8 | ≥ 16 |
| | *Streptococcus* spp. (not *S. pneumoniae*) | | | ≥ 24 | 23-17 | ≤ 16 | ≤ 0.25 | ≥ 8 |
| | *Listeria monocytogenes* | | | ≥ 20 | - | ≤ 19 | ≤ 2 | ≥ 4 |
| | *Mannheimia haemolytica* | | | ≥ 26 | 25-20 | ≤ 19 | ≤ 0.25 | ≥ 1 |
| c) | Apramycin | 40 µg | APRAM | ≥ 23 | 22-20 | ≤ 19 | ≤ 4 | ≥ 16 |
| c) e) h) | Cefadroxil | 30 µg | CDX30 | ≥ 23 | 22-20 | ≤ 19 | ≤ 8 | ≥ 32 |
| e) h) | Cefazolin | 30 µg | CFZ30 | ≥ 23 | 22-20 | ≤ 19 | ≤ 2 | ≥ 8 |
| f) | Cefoxitin | 30 µg | CFO30 | | | | | |
| | *S. aureus* | | | ≥ 22 | - | ≤ 21 | OxaS | MecA pos |
| | Coag. neg. staph. | | | ≥ 25 | - | ≤ 24 | OxaS | MecA pos |
| a) c) h) | Ceftriaxone (Cefoperazone, Cefovecin) | 30 µg | CTR30 | ≥ 23 | 22-20 | ≤ 19 | ≤ 1 | ≥ 4 |
| h) | Cefpodoxime (dogs) | 10 µg | CPD10 | ≥ 21 | 20-18 | ≤ 17 | ≤ 2 | ≥ 8 |
| c) h) | Cefquinome | 30 µg | CFQUI | ≥ 23 | 22-20 | ≤ 19 | ≤ 2 | ≥ 8 |
| h) | Ceftiofur | 30 µg | CFTIO | ≥ 21 | 20-18 | ≤ 17 | ≤ 2 | ≥ 8 |
| c) h) | Cefuroxime | 30 µg | CXM30 | ≥ 18 | 17-15 | ≤ 14 | ≤ 8 | ≥ 32 |
| c) e) h) | Cephalexin | 30 µg | CFLEX | ≥ 20 | 19-17 | ≤ 16 | ≤ 8 | ≥ 32 |
| c) e) h) | Cephalothin | 30 µg | CEP30 | ≥ 23 | 22-20 | ≤ 19 | ≤ 2 | ≥ 8 |
| | Cephapirin | 30 µg | CFP30 | ≥ 18 | 17-15 | ≤ 14 | ≤ 8 | ≥ 32 |
| | Chloramphenicol | 30 µg | CLR30 | | | | | |
| | *S. pneumoniae* | | | ≥ 21 | - | ≤ 20 | ≤ 4 | ≥ 8 |
| | *Streptococcus* spp. | | | ≥ 21 | 20-18 | ≤ 17 | ≤ 4 | ≥ 16 |
| | Other organisms | | | ≥ 18 | 17-13 | ≤ 12 | ≤ 8 | ≥ 32 |
| j) | Ciprofloxacin | 1 µg | CIPR1 | | - | | | |
| | *Salmonella spp.* | | | ≥ 30 | | < 30 | ≤ 0.06 | ≥ 0.12 |
| i) | Clindamycin | 2 µg | CLIN2 | | | | | |
| | *Staphylococcus* spp. | | | ≥ 21 | 20-15 | ≤ 14 | ≤ 0.5 | ≥ 4 |
| | Colistin | 10 µg | CO.10 | ≥ 15 | - | <14 | ≤ 2 | ≥ 2 |
| c) | Doxycycline | 30 µg | DOX30 | ≥ 14 | 13-11 | ≤ 10 | ≤ 4 | ≥ 16 |
| | Enrofloxacin | 5 µg | ENR.5 | ≥ 23 | 22-19 | ≤ 18 | ≤ 0.25 | ≥ 1 |
| | Enrofloxacin | 10 µg | ENROF | ≥ 25 | 24-21 | ≤ 20 | ≤ 0.25 | ≥ 1 |
| i) | Erythromycin | 15 µg | ERY15 | | | | | |
| | *Streptococcus* spp. | | | ≥ 21 | 30-16 | ≤ 15 | ≤ 0.25 | ≥ 1 |
| | *Staph./Enterococci* | | | ≥ 23 | 22-14 | ≤ 13 | ≤ 0.5 | ≥ 8 |
| | Florfenicol | 30 µg | FFC30 | | | | | |
| | Cattle | | | ≥ 19 | 18-15 | ≤ 14 | ≤ 2 | ≥ 8 |
| | Swine | | | ≥ 22 | 21-19 | ≤ 18 | ≤ 2 | ≥ 8 |
| | Fish pathogens | | | ≥ 28 | 27-24 | ≤ 23 | ≤ 2 | ≥ 8 |
| c) | Flumequine | 30 µg | FLUME | ≥ 20 | 19-17 | ≤ 16 | ≤ 2 | ≥ 4 |
| | Fosfomycin (U) | 200 µg | FO200 | ≥ 18 | - | < 18 | ≤ 32 | > 32 |

| | | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|---|
| c) | | Fucidin | 100 µg | FUCID | | | | | |
| | | Plain agar | | | ≥ 28 | 27-24 | ≤ 23 | ≤ 1 | ≥ 4 |
| | | Blood agar | | | ≥ 26 | 25-23 | ≤ 22 | ≤ 1 | ≥ 4 |
| | | Fucidin | 10 µg | FUC10 | ≥ 21 | - | ≤ 20 | ≤ 1 | ≥ 1 |
| c) | | Furazolidone | 50 µg | FURAZ | ≥ 23 | 22-20 | ≤ 19 | ≤ 4 | ≥ 8 |
| | | Gentamicin | 10 µg | GEN10 | ≥ 16 | 15-13 | ≤ 12 | ≤ 2 | ≥ 8 |
| l) | | Gentamicin | 250 µg | GN250 | | | | | |
| | | *Enterococci* | | | - | - | < 14 | - | HLR |
| | | Kanamycin | 30µg | KAN30 | ≥ 18 | 17-14 | ≤ 13 | ≤ 16 | ≥ 64 |
| c) | i) | Lincomycin/Neomycin (Albiotic Forte) | 15+60 µg | LIN+N | ≥ 20 | 19-17 | ≤ 16 | ≤ 2/4 | ≥ 5/16 |
| | i) | Linco-spectin | 15+200µg | LI+SP | ≥ 20 | 19-17 | ≤ 16 | ≤ 4/32 | ≥ 16/64 |
| | | Imipenem | 10 µg | IMI10 | ≥ 16 | 15-14 | ≤ 13 | ≤ 4 | ≥ 16 |
| c) | | Marbofloxacin | 5 µg | MAR.5 | ≥ 20 | 19-15 | ≤ 14 | ≤ 1 | ≥ 4 |
| b) | c) | Metronidazole (anaerobes) | 16 µg | MTR16 | ≥ 28 | 27-24 | ≤ 23 | ≤ 4 | ≥ 8 |
| c) | | Naf-Pen-Strep | 5+2+20 µg | N+P+S | ≥ 20 | 19-17 | ≤ 16 | ≤ 1/1/4 | ≥ 2/2/16 |
| c) | | Neomycin | 120 µg | NEOMY | ≥ 23 | 22-20 | ≤ 19 | ≤ 6 | ≥ 25 |
| | | Neomycin/Oxacillin(Cloxacillin) | 60/5 µg | | ≥ 20 | 19-17 | ≤ 16 | ≤ 4/2 | ≥ 16/4 |
| c) | | Nitrofurantoin | 300 µg | NI300 | ≥ 17 | 16-15 | ≤ 14 | ≤ 32 | ≥ 128 |
| | | Nitrofurantoin | 100 µg | NI100 | ≥15 | 14-13 | ≤ 12 | ≤ 32 | ≥ 128 |
| c) | | Novobiocin | 5 µg | NOVO5 | | | | | |
| | | Blood agar | | | ≥ 13 | 12-11 | ≤ 10 | ≤ 2 | ≥ 4 |
| | | Plain agar | | | ≥ 16 | 15-14 | ≤ 13 | ≤ 2 | ≥ 4 |
| f) | | Oxacillin | **1 µg** | OXA.1 | | | | | |
| | | Coag. neg. staph. | | | ≥ 18 | - | ≤ 17 | ≤ 0.25 | ≥ 0.5 |
| | | *S. intermedius (pseud)* | | | - | - | - | - | - |
| | | *S. pneumoniae* (penicillin) | | | ≥ 20 | - | - | ≤ 0.06 | ≥ 2 |
| c) | | Oxolinic acid | 10 µg | OXOLI | ≥ 20 | 19-17 | ≤ 16 | ≤ 4 | ≥ 8 |
| | | Penicillin/Novo | 10 U + 30 µg | PEN+N | | | | | |
| | | Mastitis | | | ≥ 18 | 17-15 | ≤ 14 | ≤ 1/2 | ≥ 4/8 |
| | | Other organisms | | | ≥ 17 | - | ≤ 16 | ≤ 1/2 | ≥ 4/8 |
| | | Penicillin | 10 units | PEN10 | | | | | |
| k) | | *Staphylococcus* spp. | | | ≥ 29 | - | ≤ 28 | ≤ 0.12 | ≥ 0.25 |
| | | *Streptococcus viridans* | | | ≥ 26 | 25-13 | ≤ 12 | ≤ 0.12 | ≥ 4 |
| | | Beta haemolytic | | | ≥ 24 | - | - | ≤ 0.12 | - |
| | | *Enterococci* | | | ≥ 15 | - | ≤ 14 | ≤ 8 | ≥ 16 |
| | | Other organisms | | | ≥ 18 | 17-11 | ≤ 10 | ≤ 1 | ≥ 4 |
| | | Pirlimycin | 10 µg | PIRLI | ≥ 18 | - | ≤ 17 | ≤ 2 | ≥ 4 |
| | | Rifampicin | 5 µg | RIF5 | | | | | |
| | | *S. pneumoniae* | | | ≥ 20 | 19-17 | ≤ 16 | ≤ 1 | ≥ 4 |
| | | Enterococci | | | ≥ 20 | 19-17 | ≤ 16 | ≤ 1 | ≥ 4 |
| | | Spectinomycin (Pasteur., Haemoph.) | 200 µg | SPECT | ≥ 18 | 17-15 | ≤ 14 | ≤ 32 | ≥ 128 |
| | | Spiramycin | 200 µg | SPIRA | ≥ 26 | 25-23 | ≤ 22 | ≤ 2 | ≥ 8 |
| c) | | Streptomycins | 10 µg | STR10 | ≥ 15 | 14-12 | ≤ 11 | ≤ 6 | ≥ 25 |
| | | Streptomycin (ent. cocci) | 500 µg | ST500 | - | - | < 14 | - | HLR |
| | | Sulphonamides (U) | 240 µg | SULFA | ≥ 17 | 16-13 | ≤ 12 | ≤ 256 | ≥ 512 |
| | | Tetracyclines | 30 µg | TET30 | | | | | |
| | | *Staphylococci* | | | ≥ 19 | 18-15 | ≤ 14 | ≤ 4 | ≥ 16 |
| | | Cattle (BRD) | | | ≥ 23 | 22-19 | ≤ 18 | ≤ 2 | ≥ 8 |
| | | Swine (BRD) | | | ≥ 27 | 26-24 | ≤ 23 | ≤ 0.5 | ≥ 2 |
| | | *S. pneumoniae* | | | ≥ 15 | 14-12 | ≤ 11 | ≤ 4 | ≥ 16 |
| | | Tiamulin | 30 µg | TIAMU | | | | | |
| | | Spirochaetae | | | ≥ 28 | 27-24 | ≤ 23 | ≤ 1 | ≥ 4 |
| | | Actinobacillus | | | ≥ 11 | - | no zone | ≤ 16 | ≥ 32 |
| | | Streptococci | | | ≥ 25 | 24-19 | < 18 | ≤ 1 | ≥ 4 |
| c) | | Tiamulin+Tetra | 30+15 µg | - | ≥ 16 | 15-14 | ≤ 13 | ≤ 8/4 | ≥ 16/8 |
| | | Ticarcillin | 75 µg | TIC75 | | | | | |
| | | *Ps. aeruginosa* | | | ≥ 24 | 23-16 | ≤ 15 | ≤ 16 | ≥ 128 |
| | | Gram neg. ent. | | | ≥ 20 | 19-15 | ≤ 14 | ≤ 16 | ≥ 128 |

| | NEO-SENSITABS | POTENCY | CODE | Zone diameter in mm S | I | R | Break-points MIC µg/ml S | R |
|---|---|---|---|---|---|---|---|---|
| | Ticarcillin+Clavulanate | 75+10 µg | TIM85 | | | | | |
| | *Ps. aeruginosa* | | | ≥ 24 | 23-15 | ≤ 14 | ≤ 16/2 | ≥ 128/2 |
| | Tilmicosin | 80 µg | TILMI | | | | | |
| | Bovine RD | | | ≥ 18 | 17-15 | ≤ 14 | ≤ 8 | ≥ 32 |
| | Swine RD | | | ≥ 15 | - | ≤ 14 | ≤ 16 | ≥ 32 |
| c) | Trimethoprim | 5 µg | TRIM5 | ≥ 16 | 15-11 | ≤ 10 | ≤ 4 | ≥ 16 |
| g) | Trimethoprim+Sulfa | 1.25+23.75µ | SxT25 | | | | | |
| | *S. pneumonia, Haemophilus* | | | ≥ 19 | 18-16 | ≤ 15 | ≤ 0.5/9.5 | ≥ 4/76 |
| | Systemic infection | | | ≥ 16 | 15-11 | ≤ 10 | ≤ 0.5/9.5 | ≥ 4/76 |
| | Urine | | | ≥ 16 | 15 | ≤ 14 | ≤ 2/38 | ≥ 4/76 |
| c) | Tylosin | 150 µg | TYLOS | ≥ 26 | 25-23 | ≤ 22 | ≤ 4 | ≥ 16 |
| | Vancomycin | 30 µg | VAN30 | ≥ 17 | 16-15 | ≤ 14 | ≤ 4 | ≥ 32 |
| | Special tests | | | | | | | |
| | Cloxacillin | | CLOXA | Detection of plasmid mediated AmpC | | | | |
| | Phenylboronic acid | | BORON | Detection of AmpC and KPC beta lactamases | | | | |
| | Imipenem+EDTA | 10+750 µg | IM10E | Detection of metallo-β-lactamases | | | | |
| | Dipicolinic acid | | D.P.A | Detection of metallo-β-lactamases | | | | |
| | Kits | | | | | | | |
| | ESBL, AmpC, ESBL + AmpC Confirm kit | | | ESBL + AmpC | | | | |
| | ESBL + AmpC Screen Kit | | | ESBL + AmpC | | | | |
| | KPC, MBL, OXA-48 Confirm Kit | | | Carbapenemases | | | | |

**Remarks**:

a) Results with Ceftriaxone are valid for Cefoperazone and Cefovecin.

b) Metronidazole 16 µg is the representative of the Nitroimidazole-group, including Ronidazole, Ornidazole, Ipronidazole, and Moxnidazole. Results obtained with Metronidazole are applicable to the others.

c) MIC break-points have not yet been given by the CLSI.

d) From August 2005, the FDA no longer allows the use of Enrofloxacin for treating infections in poultry (to avoid development of resistance in *Campylobacter* spp.).

e) Results of Cephalothin susceptibility tests are used to predict susceptibility to the first generation cephalosporins, such as Cephadroxil and Cephalexin.

f) Results of Cefoxitin and Oxacillin with staphylococci are used to predict susceptibility to Cloxacillin. Cefoxitin resistant staphylococci should be reported as resistant to all beta-lactams. In case of discordant results between Cefoxitin and Oxacillin, report the strains as resistant (R). Use Cefoxitin for testing S. aureus. For non S. aureus staphylococci use Oxacillin 1 µg: R ≤ 17 mm (MIC ≥ 0.5 µg/ml)

g) The results of Trimethoprim+Sulfa can be used to predict the susceptibility of other potentiated sulphomanides with Trimethoprim.

h) For detection of ESBL (CTX-M) and AmpC beta-lactamases (CMY) in *Salmonella* spp. see user's guide **"Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs"** (www.rosco.dk) on "Detection of Beta-Lactamases" (7,9).

i) Routine screening of Clindamycin inducible resistance in staphyloccci/streptococci should be performed (double disk/induction test. Results are also valid for Lincomycin.

j) *Salmonella spp.* Resistant to Ciprofloxacin 1 µg should be reported as resistant to other fluoroquinolones (Enrofloxacin, Marbofloxacin etc.).

k) With S. aureus and Penicillin 10 U: Sharp zone edge (cliff) indicates beta-lactamase positive. Fussy zone edge (beach) indicates beta-lactamase negative.

l) High level Aminoglycoside resistance if zone is < 14 mm.

**Staphylococci in animals**

a) Screening of beta-lactamase production in S. aureus: Look at the inhibition zone around Penicillin 10 U Neo-Sensitabs. If the zone edge is sharp (cliff) the isolate produces beta lactamase. If the zone edge is fuzzy (beach) the isolate is beta-lactamase negative. This test is only valid for Staphylococcus aureus.

b) Screening for Methicillin (Oxacillin) resistance: Test S. aureus using both Cefoxitin 30 µg and Oxacillin 1 µg Neo-Sensitabs. In case of discordant results between Cefoxitin and Oxacillin, report the strain as resistant. For non- S. aureus staphylococci use Oxacillin 1 µg Neo-Sensitabs. R <= 17 mm (MIC >= 0.5 µg/ml).

c) Test for Clindamycin induction: Place Clindamycin 2 µg and Erythromycin 15 µg Neo-sensitabs 20 mm (edge to edge) apart from each other (for staphylococci). For streptococci the distance should be 12 mm. Flattening of the Clindamycin zone adjacent to Erythromycin 15 µg indicates inducible Clindamycin resistance. Result is reported as Clindamycin R.

d) MRSA and similar:

Bemis et al. (11) found that the Cefoxitin disk diffusion test had low sensitivity for detection of Oxacillin resistance in members of the S. intermedins (*S. intermedius, S. schleiferi subsp schleiferi* and *S. schleiferi subsp coagulens*). Oxacillin disk diffusion had a high sensitivity and specificity for detecting *mecA* mediated Oxacillin resistance.

Perreten et al. (12) describe the spread of Methicillin resistant *S. pseudointermedius* in Europe and North America. Besides MR, the isolates showed resistance to Trimethoprim 90.3%, Gentamicin 88.3%, Streptomycin 90.3%, Macrolides 89.3%, Floroquinolones 87.4%.

High occurrence of MRSA and Methicillin resistant S pseudintermedius ( 15,16) was found in horses and small animals respectively.

Petersen et al (24) mention that the recent discovery of human and bovine MRSA isolates carrying a new mecA gene (now designated mecC) caused concern, because they are not detected by conventional confirmatory tests for MRSA. They conclude, that that mecC carrying MRSA can be exchanged between humans and ruminants.

Walther et al (25) found that the new MRSA variant (mec C) is not restricted to ruminants or humans, but it was also found in companion animals (dogs and cats). The isolates were resistant to 6 µg/ml Cefoxitin, but were not detected by the mecA test.

Vanderhaegen et al (26) isolated MR non-aureus staphylococci from bovines. They were S. sciuri, S. lentus and S. fleuretti. Both mecA and mecC genes were detected. Cefoxitin MIC was a poor indicator

for mecA mediated resistance in non-aureus staphylococci from animals. Both Oxacillin and Cefoxitin should be tested. Resistance to one or both indicates Methicillin resistance.

**Enterococci**

Detection of High Level resistance:
Use Gentamicin 250 µg Gentamicin and Streptomycin 500 µg Neo-Sensitabs.

Enterococci showing zones of inhibition < 14 mm around Gentamicin 250 µg Neo-sensitabs should be reported as High Level Resistant to Gentamicin (no synergism with penicillins).

Enterococci showing zones of inhibition < 14 mm with Streptomycin 500 µg Neo-Sensitabs should be reported as High level resistant to Streptomycin (no synergism with penicillins).

**ESBL, AmpC and carbapenemases**

Vo et al. (13) found that 17 Ceftiofur resistant isolates from horses (4 *E. coli* and *3 K. pneumoniae)* were multidrug resistant. 5 produced ESBL and 1 produced AmpC with integrons in 6 isolates.

Hopkins et al. (14) report a marked increase of *Salmonella enterica serovar* resistant to Ampicillin, Streptomycin, Sulfonamides and Tetracycline, has been noted in food-borne infections, and in pigs, pig meat in several European countries. To prevent a global epidemic of these newly emerging clones or strains, as occurred with *S. tiphimurium* DT104, intervention strategies are needed as soon as possible.

CTX-M , ESBL enzymes have been found in egg, bovine mastitis, raw chicken, broiler chickens and turkeys in the UK and Europe (17,18,19,20).

Madec et al (21) found multidrug resistant Salmonella enterica serovar tiphimurium DT104 in cattle in France. They were Ceftiofur resistant and Cefoxitin susceptible, showing a typical Cephalosporin/Clavulanate synergy, indicating ESBL production.

Leverstein et al (22) conclude that intestinal carriage with ESBL-producing bacteria in food-producing animals and contamination of retail meat may contribute to increased incidences of infections with ESBL-producing bacteria in humans. Transmission of ESBL genes from poultry to humans takes place most likely through the food chain.

Fischer et al (23) isolated an E. coli producing VIM-1 (MBL) carbapenemase on a pig farm and conclude that the prevalence of carbapenemases in bacteria from livestock is probably underestimated.

Dierikx et al (27) found a high prevalence (6.8%) of ESBL/AmpC producing E. coli at Dutel broiler farms and a high prevalence of ESBL/AmpC in E. coli from farmers. Most common genes were CTX-M1, SHV-12 (ESBLs) and CMY-2 (AmpC).

Sunde et al (28) reports the first isolate (E. coli 1248) of animal origin (broiler) detected in Norway, with reduced susceptibility to cephalosporins. The ESBL was detected using Ceftazidime and Cefepime Neo-sensitabs with and without Clavulanate. The detection was unexpected and, probably, it has been part of the bacterial flora of animals taken to Norway for breeding purposes.

Cottell et al (29) detected ESBL, CTX-M32 in 29 of 88 steers over a 26 day period. Besides, CTX-M positive bacteria were found in feces in greater numbers than previously reported in the United States. The authors fear that the CTX-M genes may spread among animals.

**Detection of ESBL, AmpC and carbapenemases in animals**

Screening:

Enterobacteriaceae showing the following zones of inhibition:

Cefpodoxime 10 µg Neo-Sensitabs : <= 17 mm and/or

Cefotaxime 30 µg or Cefquinome 30 uµ Neo-Sensitabs : <= 27 mm and/or

Ceftazidime 30 µg and/or Ceftiofur 30 uµ Neo-Sensitabs : <= 22 mm

Should be suspected of possessing an **ESBL** and should be tested to confirm it.

Isolates producing zones of inhibition <= 16 around Cefoxitin 30 µg Neo-Sensitabs should be suspected of possessing an **AmpC** beta-lactamase and should be tested to confirm it.
Use the ESBL + AmpC Screen kit or the Total ESBL + AmpC Confirm Kit from Rosco Diagnostica.

Isolates producing zones of inhibition < 23 mm around Imipenem 10 µg Neo-Sensitabs should be suspected of possessing a **Carbapenemase** and should be tested to confirm it. Use the KPC, MBL OXA-48 Confirm Kit from Rosco Diagnostica.

**Quality Control of Veterinary Antibiotics**

| | | | Zone diameter in mm | | | |
|---|---|---|---|---|---|---|
| **NEO-SENSITABS** | **POTENCY** | **CODE** | ***E. coli* ATCC 25922** | ***S. aureus* ATCC 25923** | ***Ps. aeruginosa* ATCC 27853** | ***S. pneumoniae ATCC 29212*** |
| Apramycin | 40 µg | APRAM | 21-28 | 22-30 | 18-24 | - |
| Cefquinome | 30 µg | CFQUI | 28-36 | 25-33 | - | 30-38 |
| Ceftiofur | 30 µg | CFTIO | 26-31 | 27-31 | 14-18 | 30-36 |
| Enrofloxacin | 5 µg | ENR.5 | 32-40 | 27-31 | 15-19 | - |
| Florfenicol | 30 µg | FFC30 | 22-28 | 22-29 | - | 24-31 |
| Marbofloxacin | 5 µg | MAR.5 | 29-37 | 24-30 | 20-25 | - |
| Penicillin/Novo | 10/30 µg | PEN+N | - | 30-36 | - | 24-30 |
| Pirlimycin | 10 µg | PIRLI | - | 25-32 | - | - |
| Tiamulin | 30 µg | TIAMU | - | 22-27 | - | - |

**Acceptable Q.C ranges for Campylobacter jejuni ATCC 33560.**
Incubation at 36-37° C for 24 hours:

| **NEO-SENSITABS** | **POTENCY** | **CODE** | **Zone diamater in mm** |
|---|---|---|---|
| Nalidixan | 30 µg | NAL30 | 25-34 |
| Ciprofloxacin | 5 µg | CIPR5 | 32-45 |
| Erythromycin | 15 µg | ERY15 | 26-38 |

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