Antimicrobial Susceptibility Testing

IVD

INTENDED USE

Etest is a quantitative technique for determining the antimicrobial susceptibility of Gram-negative and Gram-positive aerobic bacteria such as *Enterobacteriaceae*, *Pseudomonas*, *Staphylococcus* and *Enterococcus* species and fastidious bacteria, such as anaerobes, *N. gonorrhoeae*, *S. pneumoniae*, *Streptococcus* and *Haemophilus* species. The system comprises a predefined antibiotic gradient which is used to determine the Minimum Inhibitory Concentration (MIC), in µg/mL, of different antimicrobial agents against microorganisms as tested on agar media using overnight incubation.

SUMMARY AND EXPLANATION

Current Antimicrobial Susceptibility Testing (AST) methods are based either on quantitative dilution techniques or qualitative diffusion procedures. Dilution methods are based on two-fold serial dilutions of antibiotics in broth or agar media. These methods generate the MIC value i.e. Minimum Inhibitory Concentration of a given antibiotic in µg/mL that will inhibit the growth of a particular bacterium under defined experimental conditions.

PRINCIPLES OF USE

The Etest gradient technology is based on a combination of the concepts of dilution and diffusion principles for susceptibility testing. As with other dilution methods, Etest directly quantifies antimicrobial susceptibility in terms of discrete MIC values. However, in using a predefined, stable and continuous antibiotic concentration gradient, Etest MIC values can be more precise and reproducible than results obtained from conventional procedures based on discontinuous two-fold serial dilutions.

Etest is a thin, inert and non-porous plastic strip. One side of the strip (A) carries the MIC reading scale in µg/mL and a two or three-letter code on the handle to designate the identity of the antibiotic. A predefined exponential gradient of antibiotic, dried and stabilised, is immobilised on the other side of the strip (B) with the concentration maximum at **a**, and the minimum at **b** (Figure 1). The gradient covers a continuous concentration range across 15 two-fold dilutions of a conventional MIC method.

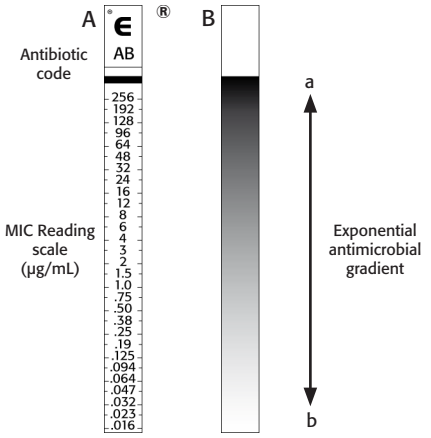


Figure 1: Etest gradient configuration

When an Etest gradient strip is applied to an inoculated agar surface, there is immediate and effective transfer of the preformed antibiotic gradient on the plastic carrier surface into the agar matrix. A stable, continuous and exponential gradient of antibiotic concentrations is formed directly underneath the strip. After incubation, whereby bacterial growth becomes visible, a symmetrical inhibition ellipse centred along the strip is seen. The MIC value is read from the scale in terms of µg/mL where the pointed end of the ellipse intersects the strip.

To obtain reproducible MICs from a gradient based system, the stability of the gradient must be maintained throughout the critical period when the position of the growth/inhibition edge for a particular bacterium/antibiotic combination is determined. Due to the stability and precision of the Etest predefined gradient, MIC values have been shown to be reproducible and equivalent to those of the CLSI® reference dilution procedures.

REAGENTS

Etest is supplied in a package of 30 or 100 test strips (depending on package format) of one antimicrobial agent.

STORAGE

Etest should always be stored according to the temperature specified on the packaging, until the given expiry date. Products can always be stored lower than the maximum temperature specified.

Etest gradient strips left over from an opened package must be kept dry. The opened package should be either re-sealed with a sealing clamp or placed in an airtight storage container with active desiccant, and stored within the temperature range stated on the label. Left-over strips in storage containers can be used until the expiry date if correctly stored and handled. Ensure that the batch number and expiry date are marked on the storage container. Protect Etest strips from moisture, heat and direct exposure to strong light at all times.

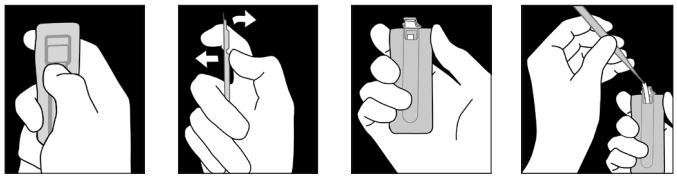
Prevent moisture from penetrating into or forming within the package or storage container. Etest strips must be kept dry with active desiccant.

HANDLING

- Before using the Etest gradient strips from an unopened package, visually inspect to ensure the package is intact. Do not use the Etest gradient strips if the package has been damaged.

- When removed from the refrigerator/freezer, allow the original package or storage container to reach room temperature before opening (+4 °C/ approx. 15 minutes, -20 °C/ approx. 30 minutes). Ensure that moisture condensing on the outer surface has evaporated completely before opening the package. Packages stored at room temperature can be used immediately.

- Opening instructions:
  - Single Pack (refer to diagram below)
    1. Hold the packaging between the thumb and the index finger, placing the thumb tip on the indented area on the back.
    2. Press forward with the thumb and back with the index finger to break open the aluminium film, ensuring that the desiccant remains in the top part of the packaging.
    3. Bend the top part back to open the packaging completely.
    4. Remove the Etest strip from the packaging using forceps or other manual applicator.



1. 2. 3. 4.

  - Blister
    - Open one blister compartment by cutting the packaging along the dotted line using scissors.
  - Foam
    - Open the packaging by cutting off one end of the aluminium pouch using scissors.

- When handling Etest strips manually, grip only the handle of the strip i.e. the area labelled E. **Do not touch the surface of the strip with the antibiotic gradient, i.e. the side opposite the MIC scale (Figure 1, B).** Strips can be placed in an applicator tray until ready to use (Figure 3). A manual applicator (e.g., Mini Grip-It, forceps or similar device) or the vacuum pen Nema C88™ (bioMérieux SA) can be used to efficiently pick up Etest strips (Figures 3 & 4). The foam cartridges carrying the Etest strips should be directly loaded onto the automatic applicator instrument Simplex C76™ (bioMérieux SA).

PRECAUTIONS AND WARNINGS

- Etest is intended for *in vitro* diagnostic use only.
- Although based on a simple procedure, Etest should only be used by trained personnel.
- Aseptic procedures and precautions against microbiological hazards should be used when handling bacterial specimens.

PROCEDURES

Materials provided

- 30 or 100 Etest strips of one antibiotic
- 1 package insert + TABLE 1 provided in the kit or downloadable from **www.biomerieux.com/techlib**

Materials required but not provided

- Agar plates (90 mm or 150 mm) with the appropriate susceptibility test media
- Inoculum suspension media
- Swabs (sterile, non-toxic and not too tightly spun), test tubes, and scissors
- Manual applicator [e.g., Mini Grip-It (bioMérieux Ref. 411200), forceps or similar device] or bioTools [Retro C80™ (bioMérieux Ref. 559803), Nema C88™ (bioMérieux Ref. 559804), Simplex C76™ (bioMérieux Ref. 559802)]
- 0.5 and 1 McFarland turbidity standards (bioMérieux Ref. 70 900) or DENSIMAT (bioMérieux Ref. 99 234)
- Incubator (35 ± 2 °C), anaerobic jar or chamber or CO_2 enriched chamber
- Quality control organisms (e.g., LyfoCults® Plus)
- Storage containers with active desiccant capsules (bioMérieux Ref. 501603, 559900, 559901 or 559902), or pouches and/or sealing clamps (bioMérieux Ref. 559809)

Medium

Ensure that the agar plate has a depth of 4.0 ± 0.5 mm, pH 7.3 ± 0.1 and fulfils quality specifications. The medium and supplements will depend on the organism groups being tested (Table 2).

Inoculum preparation

Use the inoculum guide in Table 2. Emulsify several well-isolated colonies from an overnight agar plate in a suitable suspension medium to achieve the specified inoculum turbidity by comparing to a McFarland turbidity standard. For fastidious organisms such as pneumococci, streptococci, gonococci, anaerobes and *Haemophilus* spp., use the suspension prepared in broth within 15 minutes.

Inoculation

Soak a sterile, non-toxic and not too tightly spun swab in the inoculum suspension and remove excess fluid by pressing it against the inside wall of the test tube. Remove more fluid when streaking a 90 mm plate and less for a 150 mm plate. Carefully streak the entire agar surface three times, rotating the plate 60 degrees each time to evenly distribute the inoculum. Alternatively, use Retro C80 (rota-plater, bioMérieux SA) to efficiently streak the inoculum over the agar surface. Allow excess moisture to be absorbed for approximately 15 to 20 minutes so that **the surface is completely dry before applying the Etest gradient strips**.

Notes:

1. When the inoculum and inoculation are optimal, an even confluent growth will be obtained.
2. McFarland turbidity standards do not guarantee correct number of viable cells in the suspension. Perform colony counts regularly to verify that the inoculum procedure gives the correct number of viable cells in CFU/mL. Please refer to the **QUALITY CONTROL** section.
3. Additional technical information is provided in the Etest Application Guide (EAG) available at www.biomerieux.com/techlib.

Application

Check that the inoculated agar surface is completely dry before applying Etest gradient strips.
Open the package and handle the Etest strips as described under **HANDLING**. A template can be used to optimally position Etest strips in an equidistant pattern on an agar plate. Four to six (maximum) Etest strips can be placed on a 150 mm agar plate (Figure 2a). For single MICs, one or two strips can be used on a 90 mm agar plate (Figure 2b). Strip placement is automatically optimised when using Simplex C76 (Figure 6). For organisms expected to be highly susceptible, use fewer strips per 150 mm plate and only one on a 90 mm plate.

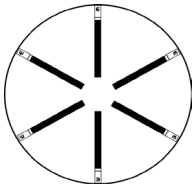


Figure 2a.
Template for 6 strips per 150 mm plate.

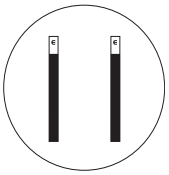


Figure 2b.
Template for 2 strips per 90 mm plate.

Etest strips can be applied to the inoculated agar surface with forceps, a manual applicator, Nema C88 (Figure 5) or Simplex C76 (Figure 6). Position the Etest gradient strip with the **MIC scale facing upwards** (towards the opening of the plate) and the concentration maximum nearest the rim of the plate (Figure 2a).

Figure 3.
Picking up an Etest strip from the tray using a Mini Grip-It

Figure 4.
Etest strips can be used directly from the cartridge with Nema C88

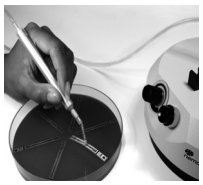


Figure 5.
Applying the Etest strip to the agar surface using Nema C88

Figure 6.
Simplex C76 automatic applicator

Ensure that the whole strip is in complete contact with the agar surface. Do not place the strip upside down as no inhibition ellipse will form since the antibiotic will not diffuse across the non-porous plastic strip. If air pockets are seen under the strip, remove them by pressing gently on the strip (without moving it) with the applicator tip or forceps, working from the lowest concentration upwards. Small bubbles will not affect results. **Once applied, the strip cannot be moved because of instantaneous release of antibiotic into the agar.**

Incubation

Incubate the agar plates in an inverted position (lid down) in stacks no higher than 5, according to conditions outlined in Table 2 and in the Etest Application Guide (EAG) available at www.biomerieux.com/techlib.

Table 2. Recommended media, inoculum and incubation [1)]

Organism group	Agar media [3)]	Inoculum		Incubation		
		Suspension	Turbidity (McFarland)	Temperature (±2 °C)	Atmosphere [8)]	Time (hours) [9)]
Aerobes	Mueller Hinton [4) 5) 6)]	0.85% NaCl (or VITEK® 2 Saline 0.45%)[10]	0.5 (1 if mucoid)	35 °C	ambient	16-20
ORSA/ ORSE	Mueller Hinton + 2% NaCl (Etest Oxacillin only)	0.85% NaCl (or VITEK 2 Saline 0.45%)[10]	0.5	35 °C	ambient	24 ORSA 48 ORSE
Anaerobes	Brucella Blood	Brucella broth or Mueller Hinton broth (or Schaedler Broth + vit. K3)[10]	1	35 °C	80-85% N_2/ 5-10% CO_2/ 10% H_2 [7)]	24-48-72 depending on the species
Haemophilus influenzae	HTM (CLSI) MHF (EUCAST)	Mueller Hinton broth or HTM broth (or Brain Heart Infusion broth (BHI))[10]	0.5 (1 if mucoid)	35 °C	5% CO_2	20-24
***Streptococcus pneumoniae* and Streptococci [2)]**	Mueller Hinton + 5% blood (CLSI) MHF (EUCAST)	Mueller Hinton broth (or VITEK 2 Saline 0.45% or BHI broth)[10]	0.5 (1 if mucoid)	35 °C	5% CO_2	20-24
Neisseria gonorrhoeae	GC-agar base + defined supplements	Mueller Hinton broth (or BHI broth)[10]	0.5	35 °C	5% CO_2	20-24

Notes:

1. Please consult Etest documents available at www.biomerieux.com/techlib for further information on specific applications.
2. Includes ß-haemolytic Streptococci groups A, B, C and G and viridans group *S. mutans*, *S. mitis*, *S. sanguis* and *S. bovis*.
3. Use well defined and high quality medium that supports good growth. The brand chosen should have good batch-to-batch reproducibility to ensure that accurate and reliable MIC values are obtained.
4. For trimethoprim and trimethoprim/sulfamethoxazole, ensure that the brand and batch of agar has a low thymine/thymidine content to minimise antagonism of the activity of trimethoprim and sulphonamides.
5. The inherent calcium content in Mueller Hinton agar may vary between brands and batch to batch. Perform quality control of agar plates on a batch to batch basis to qualify it for use, particularly for testing of daptomcyin.
6. The inherent manganese content in Mueller Hinton agar may vary between brands and batch to batch. Perform quality control of agar plates on a batch to batch basis to qualify it for use, particularly for testing of tigecycline.
7. Ensure that an efficient anaerobic system is used to achieve rapid anaerobiosis to avoid false resistant results with metronidazole.
8. When incubating fastidious organisms in 5% CO_2, the resulting pH decrease can affect the activity of macrolides, lincosamides, streptogramins, aminoglycosides, quinolones, penicillins and tetracyclines. Please be aware that differences in results can be obtained between systems that are incubated ambiently and in CO_2.
9. Ensure the agar plate is incubated for the recommended period before reading, especially for delayed expression of resistance and slow growing and fastidious organisms.
10. VITEK 2 Saline 0.45% (bioMérieux Ref. V1204), Schaedler Broth + vit. K3 (bioMérieux Ref. 42106) and Brain Heart Infusion broth (BHI) (bioMérieux Ref. 42081) have been shown to be compatible with Etest.

INTERPRETATION OF RESULTS

Reading the MIC

After the required incubation period (Table 2), and only when an even lawn of growth is distinctly visible, read the MIC value where the pointed end of the inhibition ellipse intersects the side of the strip. Do not read the plate if the culture appears mixed or if the lawn of growth is too light or too heavy; repeat the test.

Etest MIC endpoints are usually clear-cut although different growth/ inhibition patterns may be seen. Please consult the guidelines below and illustrations in the **ETEST READING GUIDE** (Figures 7 to 26).

IMPORTANT READING OBSERVATIONS

- Consult the Etest Customer Information Sheet (CIS 006) for information on the mode of action of each antibiotic (bactericidal or bacteriostatic).
- For bactericidal drugs e.g. ß-lactams, always read the MIC at the point of complete inhibition of all growth, including hazes, microcolonies and isolated colonies. Tilt the plate and/ or use a magnifying glass to carefully examine endpoints, especially for pneumococci, streptococci, enterococci, fusobacteria, *Acinetobacter* and *Stenotrophomonas* spp.
- For bacteriostatic drugs e.g. trimethoprim/sulfamethoxazole, in case of trailing endpoints, read at 80% inhibition, i.e. the first point of significant inhibition as judged by the naked eye.
- Excessively wet plates prior to inoculation, insufficient drying before applying strips and/or unevenly streaked surfaces may give non-confluent growth, jagged ellipse edges and uneven MIC intersections. Repeat the test if MIC endpoints are difficult to read.
- When macrocolonies are present within the ellipse for bactericidal agents, read all macrocolonies within 1-3 mm from the strip (consult **ETEST READING GUIDE**, Figure 21).
- When growth occurs along the entire strip i.e. no inhibition ellipse is seen, report the MIC as ≥ the highest value on the MIC scale. When the inhibition ellipse is below the strip (does not intersect the strip), report the MIC < the lowest value on the MIC scale.
- Organisms such as staphylococci, *Acinetobacter* spp., anaerobes and gonococci may be susceptible to sulbactam, tazobactam or clavulanic acid *per se*. For Etest PTc and TLc, this may result in an inhibition ellipse with an extended parallel band of inhibition alongside the strip. Extrapolate the upper elliptical curvature towards the strip to obtain the MIC (consult **ETEST READING GUIDE**, Figure 15).
- If inhibition ellipses for clindamycin, erythromycin or chloramphenicol "dip" at the endpoint, extrapolate the MIC at the initial indentation, i.e. 0.5-1 dilution above the intersection.
- For fosfomycin showing numerous (>5) macrocolonies in the inhibition ellipse, read the MIC at complete inhibition. A few (<5) colonies can be ignored.
- For quinupristin/dalfopristin and linezolid, hazy and trailing growth for staphylococci and enterococci should be read 90% inhibition as judged by the naked eye. Read isolated macrocolonies in the inhibition ellipse at complete inhibition.
- Vancomycin inhibition ellipses can be slim. Read the actual intersection at the strip and not growth "hugging" the side of the strip.

Interpretation

MIC breakpoints for defining interpretive categories as published by the CLSI®, EUCAST and/or your national reference group may be used for interpreting Etest MIC values.

Being a fully quantitative MIC method, Etest enables the laboratory to report the exact MIC value together with the interpretive category. Etest generates MIC values from a continuous scale and can give results in-between conventional two-fold dilutions i.e. half dilutions. An Etest MIC value which falls between standard two-fold dilutions must be rounded up to the next upper two-fold value before categorisation.

Example: Benzylpenicillin MIC (µg/mL) breakpoints for *Streptococcus pneumoniae* are:

S	**I**	**R**
≤ 0.06	0.12-1	≥ 2

An Etest MIC of 1 µg/mL is reported as intermediate (I) while 1.5 is rounded up to 2 µg/mL and the category reported as resistant (R).

QUALITY CONTROL

To check the performance of Etest reagents, quality of media, inoculum and procedure used, test appropriate quality control strains as outlined under **PROCEDURE**. The reagents and test procedure are considered satisfactory if MIC values obtained fall within the quality control specifications provided in **TABLE 1**.

Do not report patient results when quality control results are outside the stated QC ranges. Frequency of quality control testing should be established by the individual laboratory. Guidelines are provided in CLSI® Antimicrobial Susceptibility Testing documents M7, M11 and M100 series.

Etest quality control ranges may not be identical to CLSI specifications in all cases. Etest QC ranges are based on extensive data generated from QC testing of a large number of reagent lots over several years and include data from multi-site studies. Consult **TABLE 1** for QC specifications.

MIC results for a quality control (QC) strain that fall a half dilution below the lower QC limit should be rounded up to the next upper two-fold value before establishing QC compliance. Similarly, MIC results that are a half dilution above the upper limit show non-QC compliance.

Perform regular colony counts to verify the density of the inoculum suspension in terms of CFU/mL of viable cells. For example, dilute the inoculum suspension 1:1000 and subculture 1 µL onto the recommended agar (see Table 2). An acceptable inoculum should give approximately 100 to 500 colonies, i.e. 1 - 5 x 10^8 CFU/mL.

McFarland turbidity standards do not guarantee the correct number of viable cells in CFU/mL.

EXPECTED VALUES

Antibiotic susceptibility levels for different biological populations of bacteria are no longer predictable due to progressive development of resistance. Thus, the laboratory should use the expected MIC values of the different antibiotics for the quality control strains to ensure that testing procedures are satisfactory and that clinical results obtained are reasonably accurate.

PERFORMANCE CHARACTERISTICS

Etest performance characteristics for different antibiotic/organism groups have been established using comparative evaluations at external clinical sites and in-house testing. These studies have shown that Etest MICs correlate with the CLSI reference agar dilution and/or broth microdilution method, depending on the organism tested. Etest is considered to be substantially equivalent to the CLSI method when MIC values from both procedures show an essential agreement (EA) of ≥ 90% within ±1 dilution.
Product specifications, performance characteristics, interpretive criteria and quality control specifications are provided in **TABLE 1.**

The Etest reference database comprises more than 3000 scientific references that have demonstrated substantial equivalence between Etest and reference MIC dilution methods for a wide variety of organism groups.

IMPORTANT OBSERVATIONS

1. Indications for use (performance data) for various organism groups according to the specified recommendations are shown in **TABLE 1.**
2. Occasionally, certain antibiotic/bacterium combinations may give unusual results. In these cases, judgement of the MIC endpoint may be difficult for inexperienced personnel. However, individuals can be trained through regular use of quality control strains, Etest reading guides and comparisons with experienced personnel to correctly assess MIC endpoints.
3. Being agar based, Etest has been shown to correlate best with the reference agar dilution. Correlations have been shown with the reference broth microdilution whenever an agar dilution reference is absent.
4. As with all AST data, Etest results are *in vitro* values only and may provide an indication of the organism's potential *in vivo* susceptibility. The use of results to guide therapy selection must be the sole decision and responsibility of the attending physician who should base judgement on the particular medical history and knowledge of the patient, pharmacokinetics/pharmacodynamics of the antibiotic and clinical experience in treating infections caused by the particular bacterial pathogen with the antibiotic, dose and dosing regimen being considered.
5. For details of specific interpretive limitations and/or limitations on the clinical use of an antibiotic in various therapeutic situations, please refer to the tables and footnotes of MIC interpretive standards in the latest CLSI AST documents for dilution procedures (M7, M11 and M100 series) or EUCAST recommendations.

WASTE DISPOSAL

Unused reagents may be considered as non hazardous waste and disposed of accordingly.
Dispose of used reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

REFERENCES

1. Bolmström, A. *et al.* (1988). A Novel Technique for Direct Quantification of Antimicrobial Susceptibility of Microorganisms. ICAAC, poster 1209.
2. Baker, C. N. *et al.* (1991). Comparison of the Etest to Agar Dilution, Broth Microdilution, and Agar Diffusion Susceptibility Testing Techniques by Using a Special Challenge Set of Bacteria. *Journal of Clinical Microbiology.*
3. Brown, D. F. J. and Brown, L. (1991). Evaluation of the Etest, a novel method of quantifying antimicrobial activity. *Journal of Antimicrobial Chemotherapy.*
4. Jorgensen, J. H. *et al.* (1994). Detection of penicillin and extended spectrum cephalosporin resistance among *S. pneumoniae* clinical isolates using Etest. *Journal of Clinical Microbiology*
5. Citron D. M. *et al.* (1991). Evaluation of Etest for susceptibility testing of anaerobic bacteria. *Journal of Clinical Microbiology* .
6. Sanchez M. *et al.* (1993). Etest, an antimicrobial susceptibility testing method with broad clinical and epidemiological application. *The Antimicrobic Newsletter*.
7. Schulz J. E. *et al.* (1993). Reliability of the Etest for detection of ampicillin, vancomycin, and high-level aminoglycoside resistance in *Enterococcus* spp. *Journal of Clinical Microbiology*.
8. Baker C. N. *et al.* (1994). Optimizing testing of methicillin resistant *Staphylococcus* spp. *Diagnostic Microbiology and Infectious Disease*.
9. Tenover F. C. *et al.* (1996). Evaluation of commercial methods for determining antimicrobial susceptibility of *S. pneumoniae*. *Journal of Clinical Microbiology*.
10. Rosenblatt J. E. *et al.* (1995). Evaluation of the Etest for susceptibility testing of anaerobic bacteria. *Diagnostic Microbiology and Infectious Disease*.

Note:
Extensive Etest references based on peer reviewed literature are available from PubMed (internet).

BIBLIOGRAPHY

1. Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems; Guidance for Industry and FDA. August 2009.
2. Lorian, V. *Antibiotics in Laboratory Medicine*. 5th Ed. 2005. Williams & Wilkins, USA.
3. Murray, P.R. *et al. Manual of Clinical Microbiology*. 9th Ed. 2001. ASM Press.
4. CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. Approved Standard, M7-A (latest edition).
5. CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests of Anaerobic Bacteria*. Approved Standard, M11-A (latest edition).
6. CLSI Performance standards for antimicrobial susceptibility testing. M100-S (latest edition).

ETEST READING GUIDE

ORGANISM RELATED EFFECTS

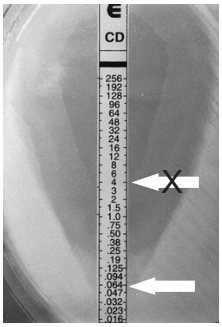


Figure 7.
Ignore swarming. MIC 0.064 µg/mL

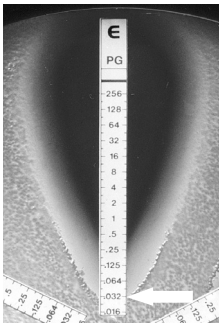


Figure 8.
Ignore haemolysis; read the inhibition of growth.
MIC 0.032 µg/mL.

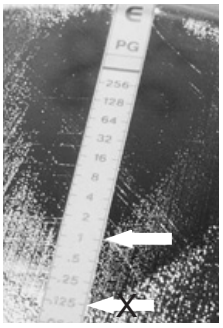


Figure 9.
Tilt plate or use a magnifying glass to see pin-point colonies and hazes, e.g. enterococci, pneumococci, fusobacteria, and *Stenotrophomonas* spp. MIC 1 µg/mL.

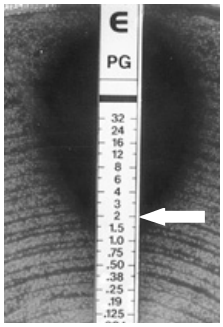


Figure 10.
Scrutinise ß-lactam endpoints for pneumococci for hazes and microcolonies.
MIC 2 µg/mL.

DRUG RELATED EFFECTS

Figure 11.
Bactericidal agents give sharp MIC endpoints.
MIC 0.064 µg/mL.

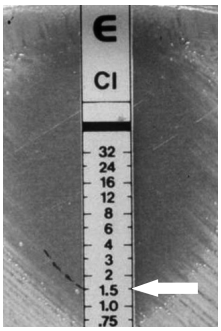


Figure 12.
Bactericidal agents; read at complete inhibition of hazes and microcolonies.
MIC 1.5 µg/mL.

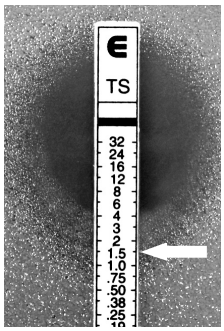


Figure 13.
Bacteriostatic agents; read at 80% inhibition.
MIC 1.5 µg/mL.

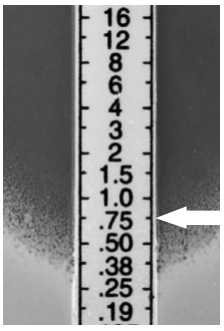


Figure 14.
Linezolid; read at 90% inhibition (ignore finer hazes and pinpoint colonies).
MIC 0.75 µg/mL.

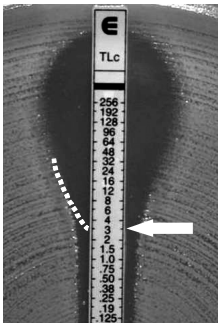


Figure 15.
ß-lactamase inhibitors e.g. tazobactam; extrapolate the upper curvature to the strip.
MIC 3 µg/mL.

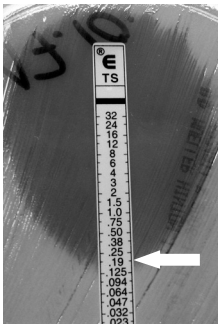


Figure 16.
Trim/sulfa; read at 80% inhibition (ignore lawn of haze within the ellipse).
Stenotrophomonas spp.
MIC 0.19 µg/mL.

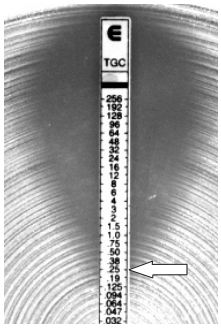


Figure 17.
Tigecycline; read at 80% inhibition (ignore trailing microcolonies or hazes).
MIC 0.25 µg/mL.

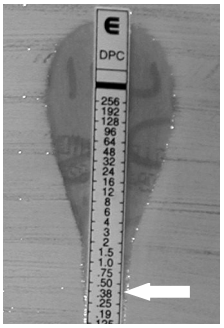


Figure 18.
Polypeptides; read at the bottom of the "dip" if colonies are absent.
MIC 0.38 µg/mL.

RESISTANCE MECHANISM RELATED EFFECTS

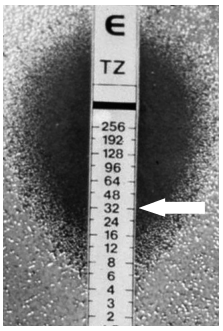


Figure 19.
Small colony variants and bactericidal agents; read at complete inhibition.
MIC 32 µg/mL.

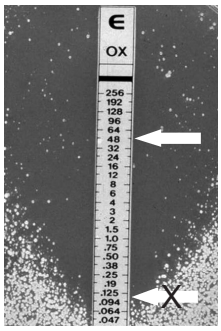


Figure 20.
Isolated colonies for oxacillin represent heteroresistant subpopulations i.e. ORSA.
MIC 48 µg/mL.

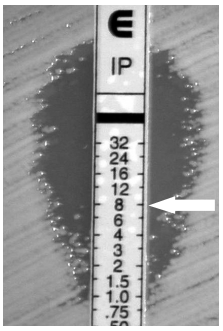


Figure 21.
Isolated colonies for carbapenems may represent resistant subpopulations e.g. KPC.
MIC 8 µg/mL.

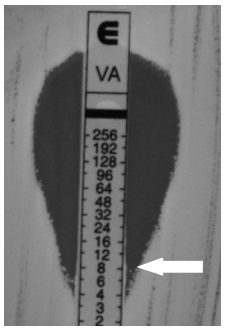


Figure 22.
Trailing growth (hazes, microcolonies, macrocolonies) represent VISA/hVISA.
MIC 8 µg/mL.

TECHNICAL AND HANDLING EFFECTS

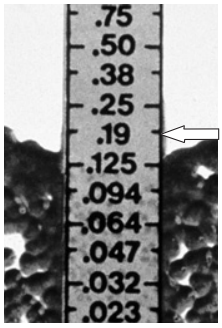


Figure 23.
Intersection in-between markings, read the upper value.
MIC 0.19 µg/mL.

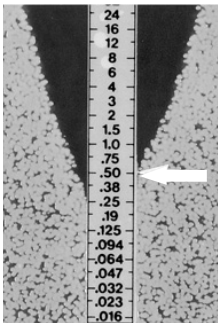


Figure 24.
Uneven intersections; read the higher value. If >1 dilution, repeat the test.
MIC 0.5 µg/mL.

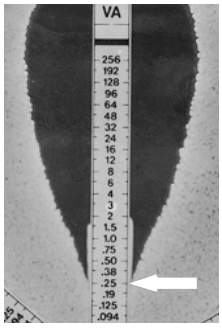


Figure 25.
Ignore a thin line of growth alongside the strip.
MIC 0.25 µg/mL.

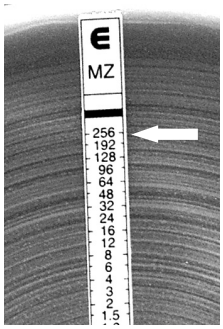


Figure 26.
Complete growth around the strip.
MIC ≥ 256 µg/mL.

WARRANTY AND DISCLAIMER

EXPRESS LIMITED WARRANTY AND DISCLAIMER

bioMérieux SA expressly warrants that Etest will determine the MIC of the antimicrobial agent on each test strip, if the procedures, precautions and important observations indicated in the package insert are strictly complied with.

bioMérieux SA makes no other warranties, expressed or implied, including the implied warranty of merchantability or fitness for particular purpose.

Any change or modification of the product instructions may affect results. bioMérieux SA shall not be liable for any damages resulting from product tampering, variance in transportation, stated storage, handling, testing procedures, precautions and other instructions of the most recently revised version of the package insert

Photos: bioMérieux SA

INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalogue number
IVD	In Vitro Diagnostic Medical Device
	Manufacturer
	Temperature limitation
	Upper limit of temperature
	Use by
LOT	Batch code
	Consult Instructions for Use
Σ	Contains sufficient for <n> tests

BIOMÉRIEUX

bioMérieux SA
Chemin de l'Orme
69280 Marcy-l'Etoile - France

RCS LYON 673 620 399
Tel. 33 (0)4 78 87 20 00
Fax 33 (0)4 78 87 20 90
www.biomerieux.com

CE