

# Etest®

Antimicrobial Susceptibility Testing  
For In Vitro Diagnostic Use

## 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.

The MIC value is not an exact entity and the "true" MIC is between the lowest concentration that inhibits the organism's growth and the next lower concentration. Even under the best of controlled conditions, a dilution test may not give the same endpoint each time it is performed. The reproducibility of the conventional dilution test is within ± 1 two-fold dilution of the endpoint. The MIC value obtained from a standardised CLSI procedure can be regarded as the reference criterion for defining the susceptibility of a microorganism.

## 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.

Although processed like the disc diffusion test, i.e. similar inoculum preparation, choice of agar media and incubation conditions, Etest is not a diffusion method and differs totally in concept from conventional disc methods. The Etest antimicrobial concentration gradient is preformed, predefined and stable, and is not dependent on diffusion.

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 surface 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.

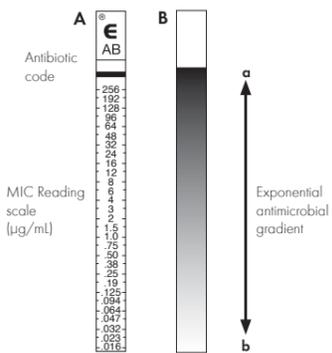


Figure 1: Etest gradient configuration

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 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 100 or 30 (some reagents) test strips of one antimicrobial agent.

## STORAGE

All packages must be stored either at controlled room temperature (18-22 °C), in a refrigerator (4-8 °C) or freezer (-18 -22 °C) as specified on the product label, until the given expiry date.

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 colour indicating desiccant, and stored at the temperature stated on the label or at -20 °C. 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.

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

## 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.

Allow the original package or storage container to reach room temperature before opening (+4 °C/ approx. 30 minutes, -20 °C/ approx. 60 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.

Open the package by carefully cutting off the top of one blister compartment, or across the top of the foil pouch. 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 Etest applicator tray until ready to use (Figure 3). The vacuum pen Nema C88™ (AB BIODISK) can be used to efficiently apply Etest strips either from the applicator tray or the original foam cartridge (Figure 4). The foam cartridges carrying the Etest strips should be directly loaded onto the automatic applicator instrument Simplex C76™ (AB BIODISK).

## 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.
- Etest should be used strictly according to the procedures described herein.
- Aseptic procedures and precautions against microbiological hazards should be used when handling bacterial specimens.

## PROCEDURES

### Materials provided

- 100 or 30 Etest strips of one antibiotic
- 1 package insert
- 1 desiccant capsule
- Table 2 (separate enclosure)

### Materials required but not provided

- Agar plates (90 mm or 150 mm) with the appropriate susceptibility test media (Table 1)
- Inoculum suspension media (Table 1)
- Swabs (sterile, non-toxic and not too tightly spun), test tubes, and scissors
- Forceps, Etest manual applicator or Biotools™ (Retro C80™, Nema C88, Simplex C76)
- McFarland 0.5 and 1 turbidity standards
- Incubator (35 ± 2 °C), anaerobic jar or chamber or CO<sub>2</sub> enriched chamber (Table 1)
- Quality control organisms
- Storage containers with colour indicating desiccant capsules, or pouches and/or sealing clamps
- Additional technical information from [www.abbiotest.com/](http://www.abbiotest.com/) Etest Technical Manual

### Agar 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 1). Additional technical information on media can be obtained from [www.abbiotest.com/](http://www.abbiotest.com/).

## Inoculum preparation

Use the inoculum guide in TABLE 1. 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 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) 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:

- When the inoculum and inoculation are optimal, an even confluent growth will be obtained.
- 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.

## 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.

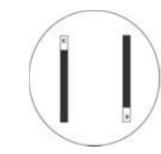


Figure 2b. Template for 2 strips per 90 mm plate.

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 a gradient strip from the tray using a manual applicator



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



Figure 5. Applying the gradient 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 1.

Table 1. Recommended media, inoculum and incubation<sup>1)</sup>.

| Organism group  | Agar media                         | Inoculum                         |                       | Incubation           |  |                                   |
|---|------------------------------------|----------------------------------|-----------------------|----------------------|--|-----------------------------------|
|   |                                    | Suspension                       | Turbidity (McFarland) | Temperature (± 2 °C) | Atmosphere   | Time (hours)                      |
| Aerobes   | Mueller Hinton                     | 0.85% NaCl                       | 0.5 (1 if mucoid)     | 35 °C                | ambient  | 16-20                             |
| ORSA/ ORSE  | Mueller Hinton + 2% NaCl           | 0.85% NaCl                       | 0.5                   | 35 °C                | ambient  | 24 ORSA<br>48 ORSE                |
| Anaerobes   | Brucella Blood                     | Brucella or Mueller Hinton broth | 1                     | 35 °C                | 80-85% N <sub>2</sub> /5-10% CO <sub>2</sub> /10% H <sub>2</sub> | 24-48-72 depending on the species |
| <i>Haemophilus influenzae</i>   | HTM                                | Mueller Hinton or HTM broth      | 0.5 (1 if mucoid)     | 35 °C                | 5% CO <sub>2</sub>   | 20-24                             |
| <i>Streptococcus pneumoniae</i> and <i>Streptococci</i> <sup>2)</sup> | Mueller Hinton + 5% sheep blood    | Mueller Hinton broth             | 0.5 (1 if mucoid)     | 35 °C                | 5% CO <sub>2</sub>   | 20-24                             |
| <i>Neisseria gonorrhoeae</i>  | GC-agar base + defined supplements | Mueller Hinton broth             | 0.5                   | 35 °C                | 5% CO <sub>2</sub>   | 20-24                             |

## Notes:

- Please consult Etest Technical Manual for further information on specific applications.
- Includes β-haemolytic *Streptococci* groups A, B, C and G and viridans group *S. mutans*, *S. mitis*, *S. sanguis* and *S. bovis*.
- 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.
- 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.
- 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 daptomycin.
- Ensure that an efficient anaerobic system is used to achieve rapid anaerobiosis to avoid false resistant results with metronidazole.
- Ensure the agar plate is incubated for the recommended period before reading, especially for delayed expression of resistance and slow growing and fastidious organisms.

## INTERPRETATION OF RESULTS

### Reading the MIC

After the required incubation period (Table 1), and only when an even lawn of growth is distinctly visible, read the MIC value where the edge 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 1 to 20) (next page).

## IMPORTANT READING OBSERVATIONS

- For bactericidal drugs e.g. β-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, read trailing endpoints 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 15).
- 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 9).
- 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. When zone edges are hazy, read the MIC at 80% inhibition.
- 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, USA 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).

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.

## 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

(TABLE 2, separate enclosure).

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 2 for QC specifications.

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 (Table 1). An acceptable inoculum should give approximately 100 to 500 colonies, i.e.  $1$  to  $5 \times 10^8$  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 in essential agreement (EA) with the CLSI method when MIC values from both procedures show an EA of  $\geq 90\%$  within  $\pm 1$  dilution.

Product specifications, performance characteristics (organism groups cleared for clinical use), interpretive criteria, quality control specifications, and limitations are provided in TABLE 2.

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 2.
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).

#### 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 Antimicrobial 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. FDA Draft, Review Criteria for Assessment of Antimicrobial Susceptibility Testing Devices. Food and Drug Administration, Division of Clinical Laboratory Devices, May 1991, revised February 2003.
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, 2006. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. Approved Standard, M7-A7.
5. CLSI, 2007. *Methods for dilution antimicrobial susceptibility tests of anaerobic bacteria*. Approved Standard, M11-A7.
6. CLSI Performance standards for antimicrobial susceptibility testing. M100 latest series.

## ESTEST READING GUIDE

### ORGANISM RELATED EFFECTS

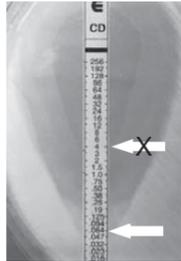


Figure 1. Ignore swarming. MIC 0.064 µg/mL

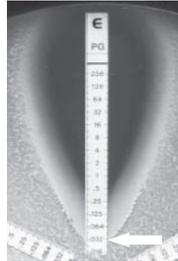


Figure 2. Ignore haemolysis; read the inhibition of growth. MIC 0.032 µg/mL

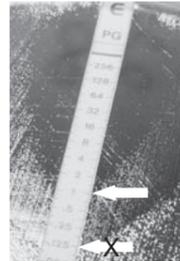


Figure 3. Tilt plate or use a magnifying glass to see pin-point colonies and hazes, e.g. enterococci, pneumococci, fusobacteria, and *Stenotrophomonas* spp. MIC 1 µg/mL

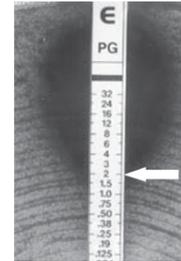


Figure 4. Scrutinise β-lactam endpoints for pneumococci for hazes and microcolonies. MIC 2 µg/mL

### DRUG RELATED EFFECTS



Figure 5. Bactericidal agents give sharp MIC endpoints. MIC 0.064 µg/mL

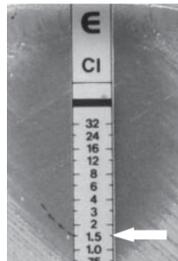


Figure 6. Bactericidal agents; read at complete inhibition of hazes and microcolonies. MIC 1.5 µg/mL

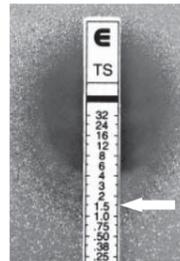


Figure 7. Bacteriostatic agents; read at 80% inhibition. MIC 1.5 µg/mL

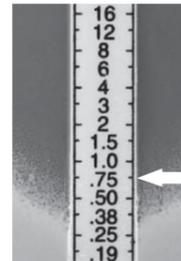


Figure 8. Linezolid; read at 90% inhibition (ignore finer hazes and pinpoint colonies). MIC 0.75 µg/mL

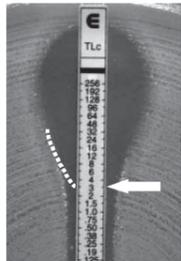


Figure 9. β-lactamase inhibitors e.g. tazobactam; extrapolate the upper curvature to the strip. MIC 3 µg/mL

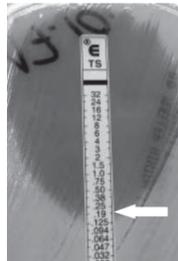


Figure 10. Trim/sulfa; read at 80% inhibition (ignore lawn of hazes within the ellipse). *Stenotrophomonas* spp. MIC 0.19 µg/mL

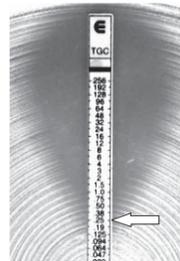


Figure 11. Tigecycline; read at 80% inhibition (ignore trailing microcolonies or hazes). MIC 0.25 µg/mL



Figure 12. Polypeptides; read at the bottom of the "dip" if colonies are absent. MIC 0.38 µg/mL

### RESISTANCE MECHANISM RELATED EFFECTS

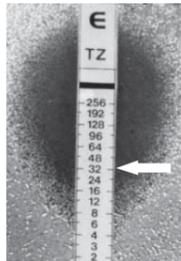


Figure 13. Small colony variants and bactericidal agents; read at complete inhibition. MIC 32 µg/mL

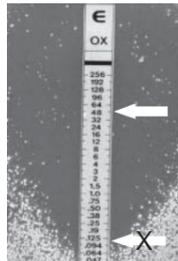


Figure 14. Isolated colonies for oxacillin represent hetero-resistant subpopulations i.e. ORSA. MIC 48 µg/mL

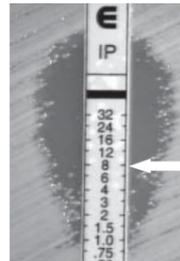


Figure 15. Isolated colonies for carbapenems may represent resistant subpopulations e.g. KPC. MIC 8 µg/mL

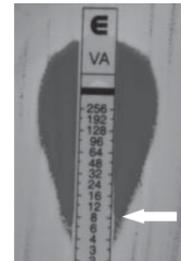


Figure 16. Trailing growth (hazes, microcolonies, macrocolonies) represent VISA/hVISA. MIC 8 µg/mL

### TECHNICAL AND HANDLING EFFECTS

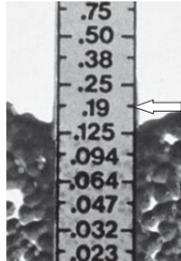


Figure 17. Intersection between markings; read the upper value. MIC 0.19 µg/mL

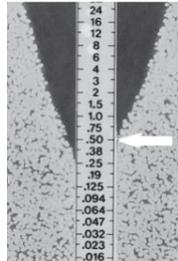


Figure 18. Uneven intersections; read the higher value. If  $>1$  dilution, repeat the test. MIC 0.5 µg/mL

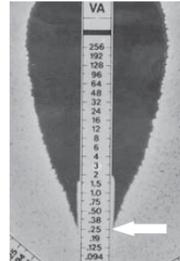


Figure 19. Ignore a thin line of growth alongside the strip. MIC 0.25 µg/mL

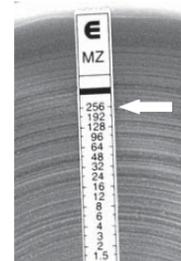


Figure 20. Complete growth around the whole strip edge. MIC  $\geq 256$  µg/mL

## WARRANTY AND DISCLAIMER

EXPRESS LIMITED WARRANTY AND DISCLAIMER

AB BIODISK expressly warrants that Etest will determine the MIC of the antimicrobial agent on each test strip, if the procedures, precautions and limitations indicated in this package insert are strictly complied with. If the test strip does not do so, AB BIODISK shall refund the cost of the product or replace the defective test strips.

**AB BIODISK makes no other warranties, expressed or implied, including the implied warranty of merchantability or fitness for particular purpose.**

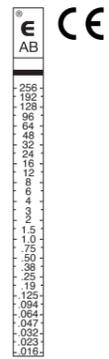
Any change or modification of the product instructions may affect results. AB BIODISK shall not be liable for any damages resulting from product tampering, variance in transportation, stated storage, handling, testing procedures, precautions and other instructions of the most recently revised version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

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# Etest®

Antimicrobial Susceptibility Testing  
For In Vitro Diagnostic Use

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