

## **E TEST Procedure**

### **For MIC Determinations of Antibiotics**

#### **I. Introduction and Clinical Significance:**

E Test is a quantitative technique to measure the in vitro activity of an antimicrobial agent against both non-fastidious Gram Negative and Gram Positive aerobic bacteria, and fastidious bacteria such as Anaerobes, Pneumococcus, and Haemophilus species. The E Test uses a predefined antibiotic gradient to determine the Minimum Inhibitory Concentration (MIC) in ug/ml of individual antibiotics against bacteria as tested on agar media by overnight incubation.

#### **II. Specimen:**

Isolated colonies (minimum of three to five).

#### **III. Materials:**

##### **A. Media and reagents**

1. Sterile saline, deionized water, suitable buffer or broth for inoculum preparation.
2. E Test strips of one antibiotic
3. Agar plates suitable for the species of bacteria being tested.
4. Quality control organisms.

##### **B. Supplies**

1. Sterile loops, swabs, test tubes, pipettes, forceps
2. McFarland 0.5 turbidity standard

##### **C. Equipment**

1. Incubator (35-37 °C), anaerobe jar or CO<sub>2</sub> enriched incubator, as appropriate per organism
2. Freezer, -20 °C to store E Test strips

#### **IV. Quality Control**

##### **A. QC Strains: Organisms**

1. *Escherichia coli* **ATCC 25922**
2. *Staphylococcus aureus* **ATCC 29213**
3. *Streptococcus pneumoniae* **ATCC 49619**

B. Additional controls

1. Growth controls: Purity plate must show good growth without any contaminating organisms present
2. Frequency of testing: Each time a patient test is performed, with the exception of *Streptococcus pneumoniae*
3. Weekly QC is done on *Streptococcus pneumoniae*
4. QC ranges are within acceptable limits: MIC's of E Tests for QC strains are with acceptable ranges.

V. Procedure:

A. Remove appropriate E Test antibiotic from the  $-20^{\circ}\text{C}$  freezer. Allow 20 minutes for the strips to come to room temperature.

B. Inoculum preparation

Emulsify an appropriate number of isolated colonies to ensure a pure culture in a suitable suspension medium such as sensi broth. Make the turbidity equivalent to a 0.5 McFarland standard.

C. Inoculation

1. Use a suitable Mueller Hinton agar plate for the bacterial species being tested.
2. Dip a sterile swab into the inoculum and swab the entire surface making a "lawn" of growth.
3. **Allow excess moisture to be absorbed (about 10 minutes) before applying the E Test strips. The surface must be completely dry before application.**

D. Application

1. Open the E Test package
  - a. Cut in-between compartments of the E Test strips
  - b. Cut along the broken line across the compartment
  - c. Use a pair of forceps to remove the strips from the package. Caution: There are three strips per package. Grip the handle only of the strip (area labeled "E" at the top).
  - d. Place the strip's bottom edge first against the agar surface with the MIC scale facing up. (If necessary, remove air pockets underneath the strip by gently pressing strip from bottom to top with forceps).
  - e. **Once applied, the position of the E Test strip cannot be changed because of the immediate release of antibiotics from**

**the strip into the agar.**

- f. Unused strips can be stored in the original compartment by taping the tip with scotch tape so that the compartment is airtight.
- g. Strips are stable until the expiration date printed on the compartment.

**E. Incubation**

- 1. Incubate immediately. The incubation temperature and atmosphere selected corresponds with the bacterial species being tested.
- 2. 35 °C - 16-18 hours aerobic incubator (ambient air)
- 3. 35 °C - 18-24 hours 5% CO<sub>2</sub>
- 4. Note: Enterococcus species and MRSA Staph aureus should be incubated a full 24 hours

**F. Reading the MIC**

After the required period of incubation and when bacterial growth becomes distinctly visible, read the MIC value at the point of intersection between the zone edge and the E Test strip. Then refer to the E Test two-fold dilution scale. See where the measured value falls on the scale, and round up to the next two-fold dilution on the scale. Report that value in the computer.

**VI. Results:**

Interpretation: Providing the QC is acceptable, interpret and report the MIC value using the E Test reporting guide and two-fold dilution scale.  
See Quality Control parameters on previous page.

**VII. Precautions:**

- A. When not in use, E Test strips should be protected at all times from moisture, heat, and direct exposure to strong light.
- B. Use only one strip on small Mueller Hinton plates, and up to 6 strips on large Mueller Hinton plates (15 cm plate).
- C. E Test strips are very thin and lightweight. Occasionally two or more strips may stick together. Make sure they are separated before putting them on the agar surface.
- D. Only touch the top of the strip where the "E" is located.

## **VIII. Reference:**

Insert - E Test A.B.Biodisk 1/1997

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Updates and Revisions: