APPENDIX X - E-TEST

I. Introduction

The E test (also known as the Gradinet Diffusion Method) is based on the same principle as the disk diffusion method. It is an in vitro method for quantitative antimicrobial susceptibility testing whereby a preformed antimicrobial gradient from a plastic-coated strip diffuses into an agar medium inoculated with the test organism. The MIC is read directly from a scale on the top of the strip at a point where the ellipse of organism growth inhibition intercepts the strip.

II. Materials

E-test strips (store frozen)
Mueller Hinton Agar (MH)
Mueller Hinton Blood Agar (MHBA)
Haemophilus Test Media (HTM)
Trypticase Soy Broth (TSB) (3 mL)
VITEK colorimeter
Sterile saline
Sterile swabs

III. Procedures

1. Allow E-test strips to come to room temperature before opening the container.

2. Using the Vitek colorimeter, prepare a suspension of the test organism in sterile saline equivalent to a 0.5 McFarland standard using isolated colonies. If there is not enough growth, inoculate the organism into TSB, and incubate at 35°C for 2-4 hours or until it reaches the turbidity of a 0.5 McFarland standard.
   For mucoid organisms, adjust suspension to 1 McFarland standard.

3. Using a sterile cotton swab, inoculate the organism onto an appropriate agar plate, streaking in 3 directions over the entire agar surface. For organisms that grow rapidly use MH agar. For *Haemophilus* species use HTM and for *S. pneumoniae* and viridans streptococci use MHBA. For other organisms that do not grow on MH, use MHBA.

4. Using forceps, apply the appropriate Antimicrobial strips onto the agar. Use one plate per Antimicrobial strip.

5. Incubate plates as follows:

   Haemophilus species - CO_2, 35°C x 18 hours
   Streptococcus pneumoniae - CO_2, 35°C x 20 to 24 hours
   Staphylococcus aureus and *Enterococcus* species for Methicillin and Vancomycin - O_2, 35°C x 24 hours
   Others - O_2, 35°C x 18 hours

IV. Interpretation

After incubation read the MIC value at the point of intersection between the zone edge and the E-test strip. Since E-test comprises a continuous gradient, MIC values in between two-fold dilutions can be obtained. Always round up these values to the next two-fold dilution before interpretation.

See e-test Reading Guide

For polymyxin B see etest Polymyxin B Reading

V. Quality Control

The 3 E-test strips are tested once weekly with *S. aureus* ATCC 29213. The organism is sub-cultured from the TSB slant (in fridge) to BA the day before setting up the QC.

Expected Results*:

	MIC
Penicillin	0.25-2.0 µg/mL
Ceftazidime	4.0-16.0 µg/mL
Ceftriaxone	1.0-8.0 µg/mL
Cefotaxime	1.0-4.0 ?g/mL

? As per NCCLS document M100-S15 (M7), Table 3, January 2005.

VI. Reference