KCMC Biotechnology Laboratory, Microbiology	STANDARD OPERATING PROCEDURE	Effective Date 28 Feb 2008	SOP-Number MIC.031
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Title: E-test Antimicrobial Susceptibility Test

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Annual Review	
By	**Date**

Title: **E-test Antimicrobial Susceptibility Test**

Document History:

Version Number	Reason for Changes	Date

Copies distributed to:

Name	Date

Title: E-test Antimicrobial Susceptibility Test

PURPOSE

For susceptibility testing of *Salmonella* spp. and *S. pneumoniae.*

PRINCIPLE

A standardized inoculum of a pure growth of a bacterial isolate is streaked onto specialized antimicrobial susceptibility testing agar and a graduated antimicrobial-impregnated strip is applied. After incubation under standardized conditions, the endpoint of the elliptical zone of inhibition of growth provides the minimum inhibitory concentration (MIC) in µg/mL. The MIC is compared to that in the table provided by the Clinical Laboratory Standards Institute (CLSI) for a particular antimicrobial-organism combination to determine susceptibility.

SCOPE

This Standard Operating Procedure applies to antimicrobial susceptibility testing by technical staff in the microbiology laboratory that have been trained and are competent in performing this procedure.

MATERIALS & STORAGE

Mueller Hinton Agar (MHA) for *Salmonella* spp. - Store at 2-8°C.
Mueller Hinton with 5% sheep blood (MHB) for *S. pneumoniae* – Store at 2-8°C.
18-24 hour pure culture of test and QC organisms on non-selective media such as blood agar
5mL Normal saline (Store at room temperature)
Cotton-tipped sterile swab
0.5 McFarland standard
Antimicrobial-impregnated E-strips (Store at -20°C with dessicant, equilibrate to room temperature before use)
Fine-tipped forceps
70% alcohol swab (Store at room temperature)

QUALITY CONTROL

1. **Frequency** - Each day of use
2. **Control Organisms (ATCC Strains)*:**

   For *Salmonella* spp. - *E. coli* 25922
   For *S. pneumoniae* -*S. pneumoniae* 49619

   *For QC organisms that are used at least once per week, sub from freezer vial once a week, incubate overnight, and use for QC testing. Store plate in the refrigerator in a ziplock bag, and sub from there the day before needed for the next QC to be set up. Discard the refrigerated plate after a week, and resub from the freezer vial. Do not subculture from refrigerated plate more than once.

3. **Acceptable Ranges** - See E-TEST QC SHEETS

4. **Corrective Action for Unacceptable QC Results**:
   a. Do not report any patient results for an antibiotic with unacceptable QC result.
   b. Repeat test using fresh subculture from freezer vial to make sure the correct isolate was used.
   c. If source of QC error cannot be determined from repeat testing, the antibiotic/organism combination must be tested daily for a period of time that will allow for the determination of the source of error.
5. **Documentation**
   a. Record results on ETEST QC SHEET.
   b. Complete a QC Deviation Form on all unacceptable resultsand submit it to supervisor.
   c. Supervisor will review QC results prior to reporting of results.

PREPARATION OF INOCULUM

1. Select at least five representative isolated colonies, more if small. If large, just gently touch each colony so as not to get too much of an inoculum.
2. Suspend in 1-5 ml of sterile normal saline and vortex until homogenous.
3. Compare with and adjust to a density equal to a 0.5 McFarland standard, adding either more colonies if not turbid enough, or more sterile saline from another tube if too turbid.

SELECTION OF ANTIBIOTICS

1. *S. pneumoniae*: penicillin and ceftriaxone
2. *Salmonella* sp.: ciprofloxacin and nalidixic acid

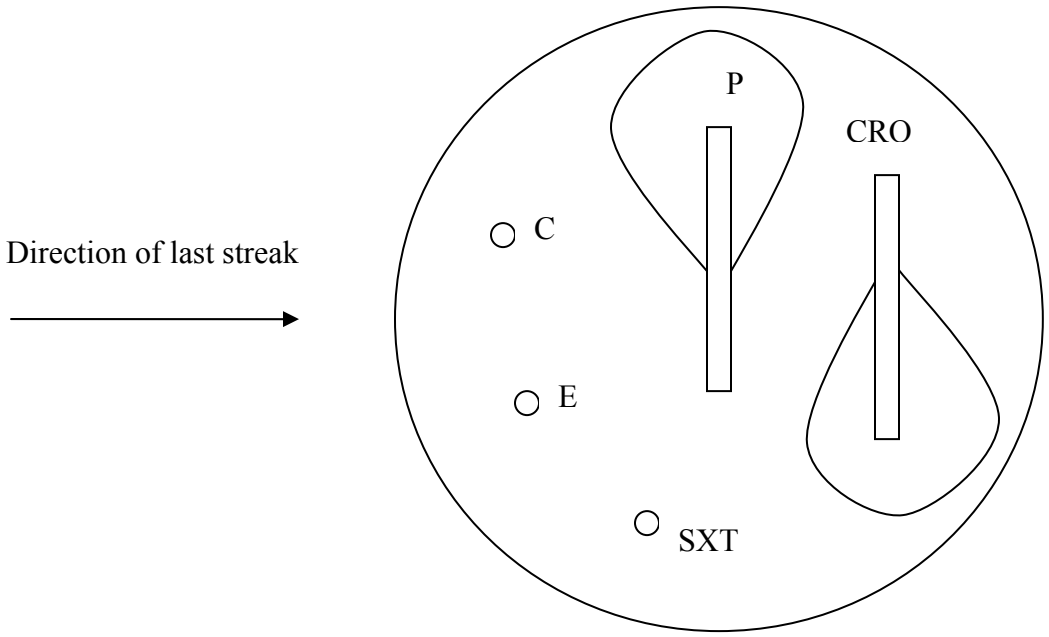
INOCULATION AND INCUBATION OF PLATES

1. Within 15 minutes of adjusting turbidity mix well, dip cotton swab in suspension, wring out excess against the wall of the tube.

2. Streak entire plate so as to achieve homogenous lawn of growth, turn plate by one-third, streak again, turn plate by one-third and streak for a third time. Finally run swab around edge of agar. Set plate with agar bed down for 10 minutes while suspension is absorbed. Draw an arrow on the plate to indicate the direction of the final streak.

3. Dispense e-tests 10 minutes after streaking to allow the suspension time to soak into the agar (but no more than 20 minutes), using fine-tipped forceps cleaned with alcohol swab. E-tests MUST be placed perpendicular to the last direction of streak or it will be difficult to read. Press E-test firmly onto agar surface, do not allow strip to move once it has been placed. Use no more than 2 E-tests per 100mm agar plate, place in opposite directions.

Placement of ETest strips on round 150mm agar plate with disks (e.g. *S. pneumoniae*):



4. Within 15 minutes of placing strips, incubate as in table below:

Organism	Agar	Incubation		
		Atmosphere	Temp	Time
Salmonella spp.	Mueller-Hinton	Aerobic	35°C	16-18hrs
S .pneumoniae	Mueller-Hinton with 5% sheep blood	5% CO_2	35°C	20-24hrs

READING ENDPOINTS

1. Read the MIC as the intersection of the elliptical zone with the E-strip, from the surface of the agar plate. When the zone ends between two gradations on the E-strip take the higher value as the MIC.

2. Read using the zone in which there is no obvious growth. Ignore the zone of α-hemolysis of agar due to *S. pneumoniae*. Presence of tiny colonies within the inhibited area may indicate that the culture is mixed, or that resistant mutants are present and this is clinically significant. If these are present, discuss with supervisor before reporting results.

REPORTING RESULTS

1. Record results and interpretation on SUSCEPTIBILITY TEST RESULT SHEET
2. Interpret results using interpretive criteria.
3. Record results on MICROBIOLOGY RESULT FORM.
4. Submit all results (including QC) to supervisor for review prior to issuing report.

REFERENCES:

Performance Standards for Antimicrobial Susceptibility Testing, M100-S16, Vol 26. No. 3. CLSI, January 2007.

APPENDIX A ETEST QC SHEET