

**KCMC Biotechnology
Laboratory, Microbiology**

**STANDARD
OPERATING
PROCEDURE**

Effective Date
27 August 2006

SOP-Number
MIC.028

Page 1 of 5

Date
10 May 2006

Title: OPTOCHIN SUSCEPTIBILITY TEST

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Approvals/Date:

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This SOP has been read and understood by:

Name	Date
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Annual Review	
By	Date

Title: OPTOCHIN SUSCEPTIBILITY TEST

Document History:

Version Number	Reason for Changes	Date

Copies distributed to:

Name	Date

Title: OPTOCHIN SUSCEPTIBILITY TEST

PURPOSE

For the differentiation of *Streptococcus pneumoniae* from other alpha hemolytic streptococci.

PRINCIPLE

Alpha hemolytic colonies are subcultured to a blood agar plate and an optochin (ethylhydrocupreine) disk is placed in the center of the subculture. After overnight incubation the plate is observed for inhibition of growth around the disk. *S. pneumoniae* is inhibited by optochin, other alpha hemolytic streptococci are not.

SCOPE

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

MATERIALS/STORAGE

Optochin Discs /Store in refrigerator
Blood Agar Plate (BAP)
Forceps

QUALITY CONTROL

Frequency: Each shipment/lot of disks prior to testing.

Control organisms/Acceptable Results:

S. pneumoniae – Zone of inhibition \geq 14 mm
Streptococcus, viridans Gp. – No zone of inhibition

Corrective actions for Unacceptable QC Results:

1. Subculture new control strain from frozen stock, subculture twice and repeat test using 5 disks.
2. If still unsatisfactory do not use discs for testing patient isolates.
3. Discard lot of disks and arrange for replacement.

Documentation:

1. Record QC results on OPTOCHIN QC sheet.
2. Document all corrective actions on unacceptable results on QC DEVIATION FORM and submit to supervisor.
3. Supervisor will review QC monthly.

PROCEDURE

1. Subculture colonies that demonstrate typical morphology of *S. pneumoniae* i.e. mucoid or smooth with depressed centers and alpha hemolytic, on to 1/2 of a BAP.

Title: OPTOCHIN SUSCEPTIBILITY TEST

2. Place optochin disc in the upper third of the streaked area.
3. Incubate plate at 35°C in 5% CO₂ overnight.

READING TEST

1. Examine for inhibition of growth around the disc.
2. Measure the size of the zone in mm.

INTERPRETATION

≥14 mm zone- S. pneumoniae

6-14 mm zone- questionable: confirm with bile solubility test or pneumococcal antigen latex agglutination test

<6 mm- negative, not S. pneumoniae

RECORDING RESULTS

1. Enter results of optochin susceptibility on POSITIVE CULTURE FORM.

NOTE

If optochin test is negative and colony has typical morphology of S. pneumoniae, perform bile solubility test or Dry Spot pneumococcal latex agglutination test to confirm.

REFERENCE:

Ruoff, KL, etal. 2003. Streptococcus. In: Manual of Clinical Microbiology. 8th Ed. ASM Press, Washington, DC.

APPENDIX A – Optochin Test QC sheet

KCMC Biotechnology Laboratory, Microbiology

STANDARD OPERATING PROCEDURE

Effective Date
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SOP-Number
MIC.028

Page 5 of 5

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Title: **OPTOCHIN SUSCEPTIBILITY TEST**

APPENDIX A

**KCMC Biotechnology Laboratory
Microbiology**

**Quality Control
OPTOCHIN TEST**

CONTROLS/ACCEPTABLE RESULTS:

Streptococcus pneumoniae - zone ≥ 14 mm (+)

Streptococcus, viridans Gp - no zone (-)

YEAR: _____

QC DATE	QC BY	DISK LOT#/ EXP DATE	<i>S. pneumoniae</i>	<i>Strep viridans</i> Gp	A/N A*

*A/NA = Acceptable/Not acceptable

DOCUMENT ALL CORRECTIVE ACTION ON QC DEVIATION FORM

Supervisor Review :							
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OPTOCHIN QC/QC SHEETS