



STANTON TERRITORIAL HEALTH AUTHORITY

Yellowknife, Northwest Territories

TITLE: Disc Diffusion-Optochin	Revision Date: 10-March-2016	Issue Date: 10-March-2014
Document Number: MIC50630	Status: Approved	
Distribution: Microbiology Test Manual	Page: 1 of 3	
Approved by: Cheryl Case, Manager of Diagnostic Services	Signed by: <i>Cheryl Case</i>	

PURPOSE:

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

SAMPLE INFORMATION:

Type	Pure, fresh culture of alpha gram positive organism
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REAGENTS and/or MEDIA:

Media	Information
Blood Agar (BAP)	<ul style="list-style-type: none">▪ Source: Oxoid▪ Store at 2 – 8°C away from direct light
Optochin disc (5µg) 6mm in diameter	<ul style="list-style-type: none">▪ Oxoid Canada▪ Storage Conditions -20 °C to 8 °C<ul style="list-style-type: none">• Store unopened cartridges in freezer• Opened cartridges can be kept in refrigerator at 2-8 °C (Oxoid Canada, 2008)

SUPPLIES:

- Sterile loop
- Tweezers
- Ruler

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SPECIAL SAFETY PRECAUTIONS:

Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods. Optochin discs have the possibility of causing allergic sensitization; apply the use of proper personal protective equipment as outlined in Optochin Disc MSDS sheet.

QUALITY CONTROL:

Quality Control is set up each day the test is performed using the following control organisms. A QC order is generated in the TQC system.

Positive: *Streptococcus pneumoniae* ATCC # 49619
Negative: *Streptococcus viridans* ATCC # 13419

- A TQC order is automatically generated to record the QC results

PROCEDURE INSTRUCTIONS:

Step	Action
Testing For Optichin Sensitivity	
1	In the plate log – Order ^TXP
2	Streak a pure culture of the organism across a BA plate
3	Apply an Optochin Disc to an area of heavy inoculum
4	Incubate in CO2 incubator for 16-18 hrs
5	Examine plate for a zone of inhibition.

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INTERPRETATION:

IF	THEN
Zone size 6-8mm	▪ <u>NOT</u> <i>Streptococcus pneumonia</i>
Zone size 9-13mm	▪ <u>CANNOT RULE-OUT</u> <i>Streptococcus pneumoniae</i> as it could be an Optochin resistant strain. ▪Repeat test. ▪Perform Bile Solubility testing – See Procedure MIC52900
Zone size greather than or equal to 14mm	▪Report <i>Streptococcus pneumoniae</i>

REFERENCES:

- American Society For Microbiology. (2004). Clinical Microbiology Procedures Handbook. In H. D. Isenberg (Ed.).
- Murray, P., Baron, E. J., Joregensen, J., Landry, M. L., & Pfaller, M. (2007). *Manual of Clinical Microbiology*. Washington, DC, USA: ASM Press.
- Oxoid Canada. (2008). Diagnostic Discs Optochin Discs. *DD0001* .
- Oxoid Canada. (2008, October 07). Diagnostic Discs-Material Safety Data Sheet. *Safety Data Sheet Diagnostic Discs* .

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	15-NOV-2010	Initial Release	M-L Dufresne
1.1	31-Dec-2013	LIS Updates	A.Darrach