PURPOSE: The optochin test is used to determine an organism's susceptibility to the chemical Optochin (ethylhydrocupreine hydrochloride) for the presumptive identification of *Streptococcus pneumoniae*.

SAMPLE INFORMATION:

Type	Few, well isolated colonies of alpha-hemolytic *Streptococci*

REAGENTS and/or MEDIA:

Type	Oxoid 5 µg Optochin Disk
Stability and Storage Requirements	• Unopened cartridges must be stored at 2°C to 8°C. • Unopened cartridges should be allowed to come to room temperature before removing them from the packaging to minimize condensation. • Opened cartridges need to be stored 2°C to 8°C, in an opaque, air tight container with a charged desiccant to protect the disks from moisture. • Open cartridges should be stored within the container in the refrigerator and be allowed to come to room temperature before opening to prevent the formation of condensation. • Once a cartridge is opened, it should be stored for no longer than a month.

PLEASE NOTE:

- **Any isolates of *Streptococcus pneumoniae* from invasive sites must be sent to NML for International Circumpolar Surveillance (ICS) program. Refer to MIC10520.**

SUPPLIES:

- Plastic Vitek tubes and caps
- 0.9% sterile saline
- Sterile swabs
- Disposable loops
- DensiCHEK Plus
- Blood agar
- Forceps
- 35° CO_2 incubator
- Small, metric ruler

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. 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.

QUALITY CONTROL:

- Quality control is performed weekly:
  - Resistant: *Streptococcus salivarius* ATCC 13419, Zone size = < 14 mm
  - Sensitive: *Streptococcus pneumoniae* ATCC 49619, Zone size = ≥ 14 mm
- A TQC order is automatically generated on Wednesdays to record the QC results.

PROCEDURE INSTRUCTIONS:

Step	Action
Performing the optochin test	
1	Using a disposable loop, select a well-isolated colony of the alpha-hemolytic organism to be tested. Alternatively, dispense 3 mL of sterile saline into a labelled plastic test tube. Pick several colonies of the alpha-hemolytic organism to be tested and prepare a suspension equivalent to a 0.5 McFarland standard.
2	Using a loop from a colony or a sterile swab from a 0.5 McFarland suspension, streak ½ a BA plate in three directions to provide confluent growth.
3	Apply the optochin disk to agar surface with forceps. Apply gentle pressure to ensure complete contact of disk with agar.
4	Incubate the plate at 35°C for 18 to 24 hours in the CO_2 incubator.
5	After incubation, read plates only if lawn of growth is confluent.
6	Use a ruler to measure the diameter of inhibition zone to the nearest millimeter, including the disk.

INTERPRETATION OF RESULTS:

IF	THEN
Zone of inhibition = < 14 mm	**Optochin = Resistant**
Zone of inhibition = 6 – 13 mm	**Optochin = Intermediate** ***Cannot rule out S.pneumoniae*** ➢ Repeat test ➢ Perform Vitek 2 GP card
Zone of inhibition = ≥ 14 mm	**Optochin = Sensitive**

LIMITATIONS:

1. *S.pneumoniae* isolates should be incubated in a CO_2-enriched environment, as some isolates will grow poorly or not at all without increased CO_2.
2. Optochin test results are presumptive. Positive results may be confirmed with more specific tests, such as the Vitek 2 GP card.
3. Occasional strains of *Streptococcus pneumoniae* that are not inhibited by optochin have been reported and strains of alpha-hemolytic *Streptococci* have been reported to form zones of approximately 10 to 12 mm when a light inoculum was used.
4. If the organism is optochin resistant, it is likely to be a nonpneumococcal alpha-hemolytic *Streptococcus*; however, rare exceptions have been reported.

REFERENCES:

- Oxoid, Optochin Test Disks product insert, 2018-07
- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	4 APR 19	Initial Release	L. Steven