National Department of Health

Title: Optochin Test

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Developed by:	**C Allen**
Reviewed by:	**J Ferguson, T Ikanofi, V Nauna**
Authorized by:	**W Porau**
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Changes to the last authorized version:

Version	Date issued	Changes

1. Purpose and Scope

This document describes the Optochin test procedure used to identify Streptococcus pneumoniae.

The optochin test detects an organism's susceptibility to the chemical optochin. The chemical tests the fragility of the bacterial cell membrane and causes S. pneumoniae to lyse due to changes in surface tension.

2. Principle/Clinical application

The optochin disk tests for the fragility of S. pneumoniae. When exposed to Optochin, Strep pneumoniae cell wall is lysed and the organism is not able to grow on the plate. Visually this is seen as a zone of inhibition around the optochin disk. The zone of inhibition must be ≥ 14 mm using a 6-mm 5ug optochin disc.

A positive presumptive identification of S. pneumoniae is made when a well-defined zone of inhibition results around the impregnated disk. Other alpha-hemolytic streptococci do not display this clear zone of inhibition when in the presence of optochin.

Optochin (ethylhydrocupreine hydrochloride) is a chemical and is completely soluble in water.
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The optochin test is widely used in the form of filter paper discs impregnated with ethylhydrocupreine hydrochloride.

3. Responsibilities

- Staff performing optochin setup require specific training and demonstrated competency.
- Staff performing Optochin are responsible for the setup, reading and recording of the optochin result.
- Staff are required to test and record Optochin positive and negative controls daily.

4. Specimen

- Test isolated colonies of alpha haemolytic Strep species (Gram positive cocci in pairs or chains and catalase negative) grown in CO2 on Blood agar for 24 hrs or less.

5. Safety

For safety aspects, please review this document G_10_Info_3 Laboratory Biosafety.

6. Equipment and Materials

- Optochin- paper discs impregnated with 5ug of ethylhydrocupreine hydrochloride or commercially available discs
- Quality Control organisms: S. pneumoniae ATCC 49619 and E. faecalis ATCC 51299
- 37C - 5% CO2 incubator or candle jar and 37C O2 incubator
- Gloves
- Loops for inoculation
- Forceps
- Blood agar plate

7. Procedure

7.1 Allow optochin disc to come to room temperature

7.2 Streak a blood agar plate with the organism to obtain confluent growth. Half plate may be used per isolate. Then continue to streak for isolation.

7.3 Aseptically, with forceps remove one optochin disc and place in the centre of the primary streaked portion of the plate. Do not place the disc too close to the edge of the plate. Gently apply pressure with forceps so that the disc adheres to the plate.

7.4 Return discs to refrigerator with desiccant.

7.5 Invert the plate and incubate at 35° C in a candle jar or 5% CO2 incubator for 18 to 24 hours.

8. Results Recording

- Record all results onto paper worksheet with registered lab number and patient identification.
- Record results into LIMS

9. Interpretation

- **Sensitive**: ≥14mm zone around the optochin disk with distinct margin.
- **Resistant:** Growth not inhibited around the disk, or < 14mm not S. pneumoniae
- Optochin sensitive alpha haemolytic colonies with characteristic morphology are *Strep. pneumoniae*

10. Quality Control

- S. pneumoniae ATCC 49619 Optochin sensitive
- E. faecalis ATCC 51299 Optochin resistant
- Record the QC results on the Bench Reagent QC Worksheet- G_90_WS_1

11. Related Documents

- Bench Reagent QC Worksheet G_90_WS_1
- Laboratory Biosafety Info Sheet G_10_Info_3

12. Reference

- DMDP Optochin Test SOP- J Morello 2014
- McFaddin J.E. Optochin disk test, Biochemical Tests for the Identification of Medical Bacteria, 2nd edition
- UK Standards for Microbiology Investigations https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality- and- consistency-in-clinical-laboratories