NATIONAL STANDARD METHOD

OPTOCHIN TEST

BSOP TP 25

Issued by Standards Unit, Evaluations and Standards Laboratory
Specialist and Reference Microbiology Division

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

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STANDARD OPERATING PROCEDURE FOR THE OPTOCHIN TEST

INTRODUCTION

Susceptibility to optochin (ethylhydrocupreine hydrochloride) is a simple and reliable method of differentiating *Streptococcus pneumoniae* from other alpha-haemolytic streptococci².

TEST PRINCIPLE

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

The optochin test is widely used in the form of filter paper discs, impregnated with ethylhydrocupreine hydrochloride which are applied directly to inoculated plates before incubation³,⁴.

1.0 SAFETY CONSIDERATIONS⁵-¹⁰

Refer to current guidance on the safe handling of all organisms and reagents documented in this SOP

All work likely to generate aerosols must be performed in a microbiological safety cabinet

The above guidance should be supplemented with local COSHH and risk assessments

Compliance with postal and transport regulations is essential

2.0 REAGENTS AND EQUIPMENT¹¹,¹²

Filter paper discs impregnated with 5µg of ethylhydrocupreine hydrochloride

Bacteriological straight wire/loop (preferably nichrome) or disposable alternative

Quality control organisms

Positive control *Streptococcus pneumoniae* NCTC 10319

Negative control *Streptococcus viridans* NCTC 10712

3.0 METHOD/PROCEDURE AND RESULTS

Pure colony

Streak a blood plate with the organism to be tested

Place an optochin disc in the centre of the inoculum

Incubate at 35-37°C for 18-24h in 5% CO₂

Examine for zones of inhibition
Specimen

Streak the specimen onto a blood plate

Place an optochin disc on the edge of the primary inoculum

Incubate at 35-37°C for 18-24h in 5% CO₂

Examine for zones of inhibition

Positive result: zone of inhibition of ≥5mm radius from the edge of the disc eg test organism is *S. pneumoniae*

Negative result: no zone of inhibition, or a zone <5mm radius from the edge of the disc eg test organism is not *S. pneumoniae*

4.0 PRECAUTIONS/LIMITATIONS OF PROCEDURE

Some “viridans” streptococci may produce a small zone of inhibition. Equivocal results should be confirmed using the bile solubility test or by the use of a commercial kit

False-negatives may be reported if cultures are incubated in high concentrations of CO₂

Occasional strains of optochin resistant *S. pneumoniae* have been reported
REFERENCES


