

Procedure for antimicrobial susceptibility testing of *Streptococcus* species (β -haemolytic) by disc diffusion

Confirm identification of isolate.
Sub-culture on sheep blood agar plate (18-20 hours)

Prepare inoculum

Prepare a **0.5 McFarland** suspension of the bacteria to be tested in sterile saline

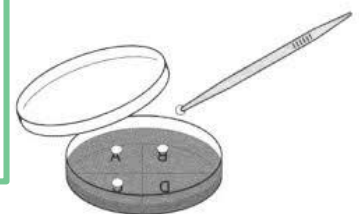
Compare prepared suspension with that of the **0.5 McFarland** standard (control) and adjust turbidity as needed with sterile saline or pure culture until correct density is achieved.



Perform **quality control** of medium as appropriate

Inoculate **Mueller Hinton Agar (MHA)** with 5% sheep blood [CLSI] **OR** MHA +5% defibrinated horse blood and 20mg/L β -NAD [EUCAST] by streaking the plate with a swab multiple times in different directions to ensure even confluent growth. Allow to dry (Maximum 10 minutes).

Allow disks and media to reach **ambient temperature** before inoculating plates and placing disks



Perform **quality control** of antimicrobial discs as appropriate.

CLSI ¹	EUCAST ²
<i>Streptococcus</i> species (β -haemolytic)	
<i>Streptococcus pneumoniae</i> ATCC®49619	

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Place discs on plate with sterile forceps/tweezers/disc dispenser. Do not move the disks once they have touched the agar surface.

Incubate

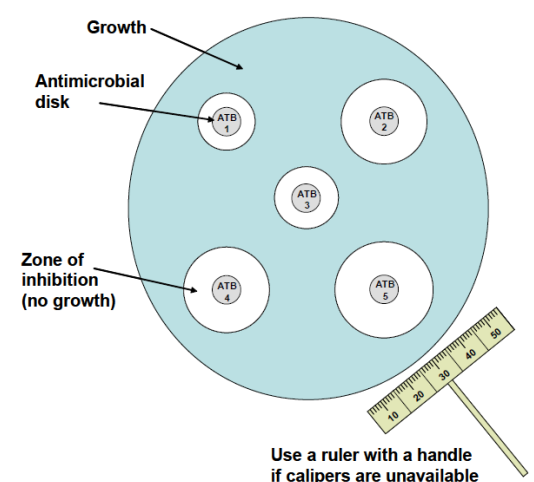
35°C±2°C; 5% CO₂; 20-24 hours



Read **quality control** strain zones of inhibition first. If within limits, read test strain. If quality control fails trouble shoot whole process.

Read zone edges at the point showing no growth against a dark background illuminated with reflected light. Measure zone diameter (mm) using ruler/calipers.

Interpret according to the latest **Clinical and Laboratory Standards Institute (CLSI) / European Committee on Antimicrobial Susceptibility Testing (EUCAST)** guidelines.



1. Clinical and Laboratory Standards Institute (2017); Performance Standards for Antimicrobial Susceptibility Testing; Twenty-fifth Information supplement. CLSI document M100-S27

2. European Committee on Antimicrobial Susceptibility Testing; Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1, 2017