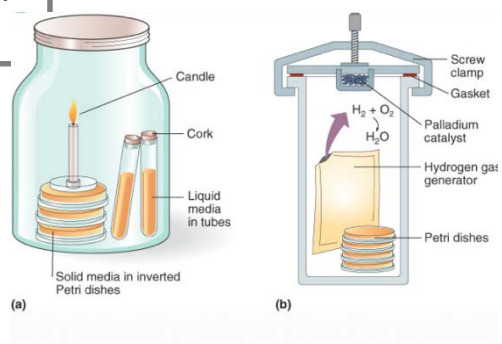
Procedure for antimicrobial susceptibility testing of *Neisseria meningitidis* by disc diffusion**

*Recommended precautions: Perform all antimicrobial susceptibility testing in a biological safety cabinet (BSC). Manipulating *N. meningitidis* outside a BSC is associated with increased risk for contracting meningococcal disease. Use appropriate personal protective equipment (PPE).

Once identification of isolate is confirmed. Sub-culture onto **supplemented chocolate agar** and incubate in a **CO_2-enhanced atmosphere** (CO_2 incubator or candle-extinction jar) for 20-24 hours[1]. *

a) Candle jar
b) Commercial CO_2 generating container



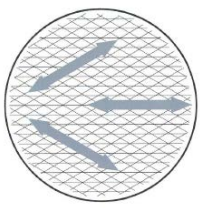
Prepare inoculum

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. Suspension must be used within 15 minutes [1,3].

Perform **quality control** of medium as appropriate

Inoculate **Mueller Hinton agar (MHA) with 5% sheep blood** by streaking the plate with a swab multiple times in different directions to ensure even confluent growth. Allow to dry (Maximum time 10 minutes).



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

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

CLSI[1]	EUCAST[2]
Neisseria meningitidis	
Streptococcus pneumoniae ATCC®49619	

EUCAST: Disc diffusion criteria for antimicrobial susceptibility testing of *N. meningitidis* have not yet been defined and MIC method should be used.[2]

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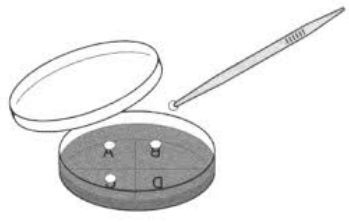
Place discs on plate with sterile forceps/tweezers/disc dispenser. Do not move the discs once they have touched the agar surface.

Incubate

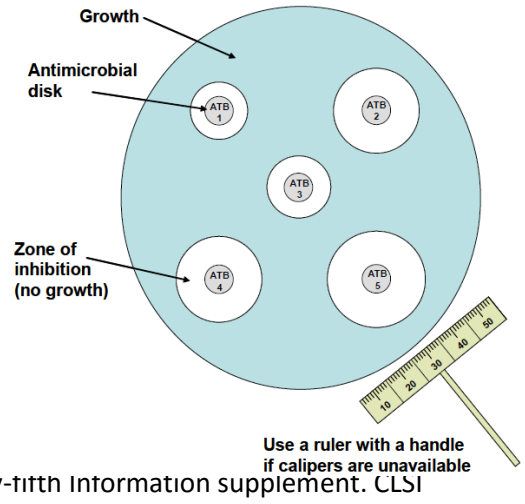
35°C±2°C; 5% CO_2; 20- 24 hours



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 as 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)** **



** Kirby-Bauer disc diffusion is the least expensive screen for antimicrobial susceptibility testing, but results can be difficult to interpret. Kirby-Bauer disc diffusion tests do not produce reliable results for ampicillin and penicillin and false intermediate or resistant results may occur with *N. meningitidis.* Results demonstrating an isolate with reduced susceptibility should be verified using minimum inhibitory concentration (MIC) test. [3]

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
3. Laboratory methods for the diagnosis of meningitis caused by *Neisseria meningitidis, Streptococcus pneumoniae* and *Haemophilus influenzae*; WHO manual; second edition (2011)