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

Once identification of isolate is confirmed. Sub-culture onto supplemented chocolate agar and incubate in a CO₂-enhanced atmosphere (CO₂ incubator or candle-extinction jar) for 20-24 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. Suspension must be used within 15 minutes 1,3.

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).

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% CO₂; 20-24 hours

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)**

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**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).

**Perform quality control of medium as appropriate

**Perform quality control of antimicrobial discs as appropriate.

<table>
<thead>
<tr>
<th>CLSI</th>
<th>EUCAST*</th>
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| *Neisseria meningitidis*
| Streptococcus pneumoniae
| ATCC®849619

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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**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

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1. Clinical and Laboratory Standards Institute (2017); Performance Standards for Antimicrobial Susceptibility Testing; Twenty-eighth informational 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