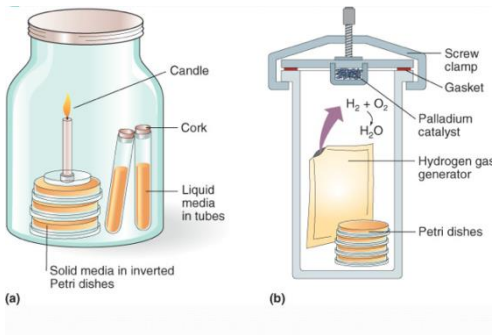
Procedure for antimicrobial susceptibility testing of *Haemophilus influenzae* by disc diffusion

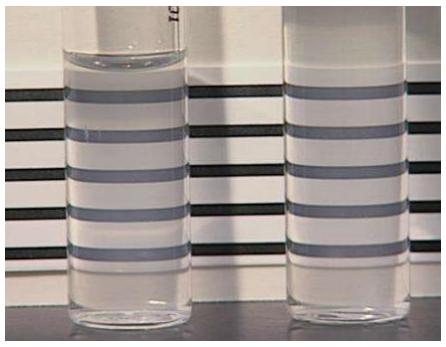
Confirm identification of isolate. Sub-culture onto **supplemented chocolate agar** and incubate in a **CO_2- enhanced atmosphere** (CO_2 incubator or candle-extinction jar).



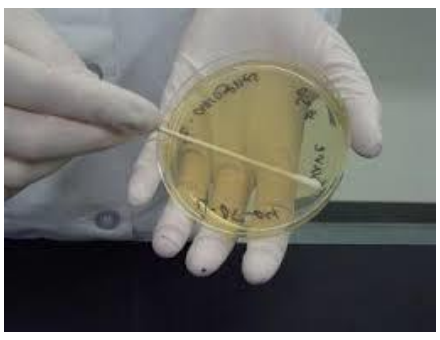
a) Candle jar
b) Commercial CO_2 generating container

Prepare inoculum

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

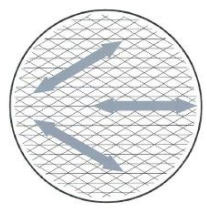


Compare prepared suspension with that of the **0.5 McFarland** standard (control) and adjust turbidity as needed with sterile saline or pure culture until correct density is achieved. Suspension must be used within 15 minutes [1,3].



Perform **quality control** of medium as appropriate

Inoculate **Haemophilus Test Medium (HTM) [CLSI guidelines] OR Mueller Hinton agar + 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 time 10 minutes).



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

CLSI[1]	EUCAST[2]
Haemophilus influenzae	
Haemophilus influenzae ATCC®49766	
Haemophilus influenzae ATCC®49247	
Escherichia coli ATCC®35218	*Staphylococcus aureus* ATCC®29213

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

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; 16-18 hours

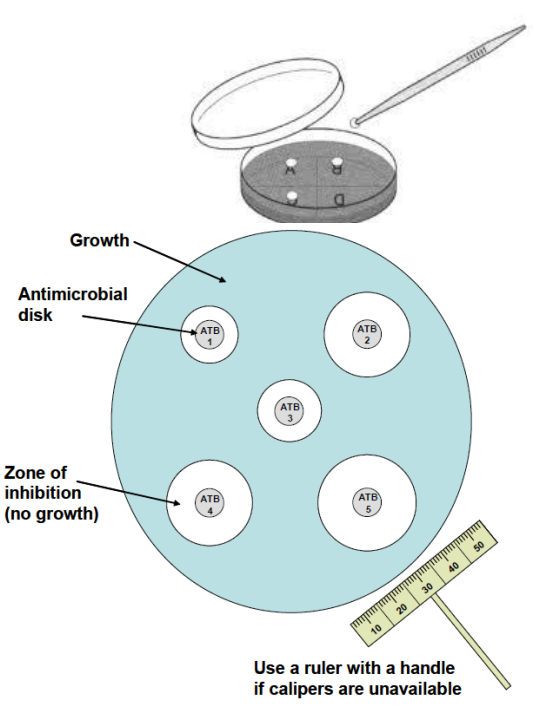
Read **quality control** strain zones of inhibition first. If within limits, read test strain. If quality control fails trouble shoot whole process.

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.

Record and report findings.

A rapid β-lactamase test yields clinically relevant information earlier than the results of the antimicrobial susceptibility testing so it should be performed as soon as a *H.influenzae* is identified. **Nitrocefin-based test are the preferred method, chromogenic** cephalosporin, which releases a red compound on hydrolysis by a β-lactamase[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)