**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 CO₂-enhanced atmosphere (CO₂ incubator or candle-extinction jar).

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.

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).

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; 16-18 hours

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.

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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