Standard Operating Procedure on Antimicrobial Susceptibility Testing Using Kirby–Bauer Disc Diffusion Method

Department of Microbiology

Faculty of Medicine

UWUSL

Standard Operating Procedure (SOP)
Antimicrobial Susceptibility Testing Using
Kirby–Bauer Disc Diffusion Method

Title: Determination of Antimicrobial Susceptibility of Bacterial Isolates Using Kirby–Bauer Disc Diffusion Technique

Issued By: Faculty of Medicine, Uva Wellassa University of Sri Lanka

(1) Purpose

To establish a standardized procedure for performing antimicrobial susceptibility testing using the Kirby–Bauer disc diffusion method during microbiology practical sessions to determine the susceptibility or resistance of bacterial isolates to selected antimicrobial agents.

(2) Scope

This SOP applies to all medical students, academic staff, and laboratory personnel involved in microbiology practical sessions, including:

- Preparation of bacterial inoculum
- Performing Kirby–Bauer disc diffusion testing
- Measuring inhibition zones
- Interpretation of antimicrobial susceptibility results

(3) Responsibilities

(3.1) Students

- Follow the correct antimicrobial susceptibility testing procedure
- Handle bacterial cultures, antibiotic discs, and equipment safely
- Accurately measure and record inhibition zones
- Interpret results according to provided guidelines

(3.2) Demonstrators / Lecturers

- Supervise the practical procedure
- Ensure correct inoculum preparation and testing technique
- Assist students in interpretation of results

(3.3) Laboratory Staff

- Provide required materials, reagents, and equipment
- Ensure proper storage of antimicrobial discs and culture media
- Maintain laboratory safety standards

(4) Principle

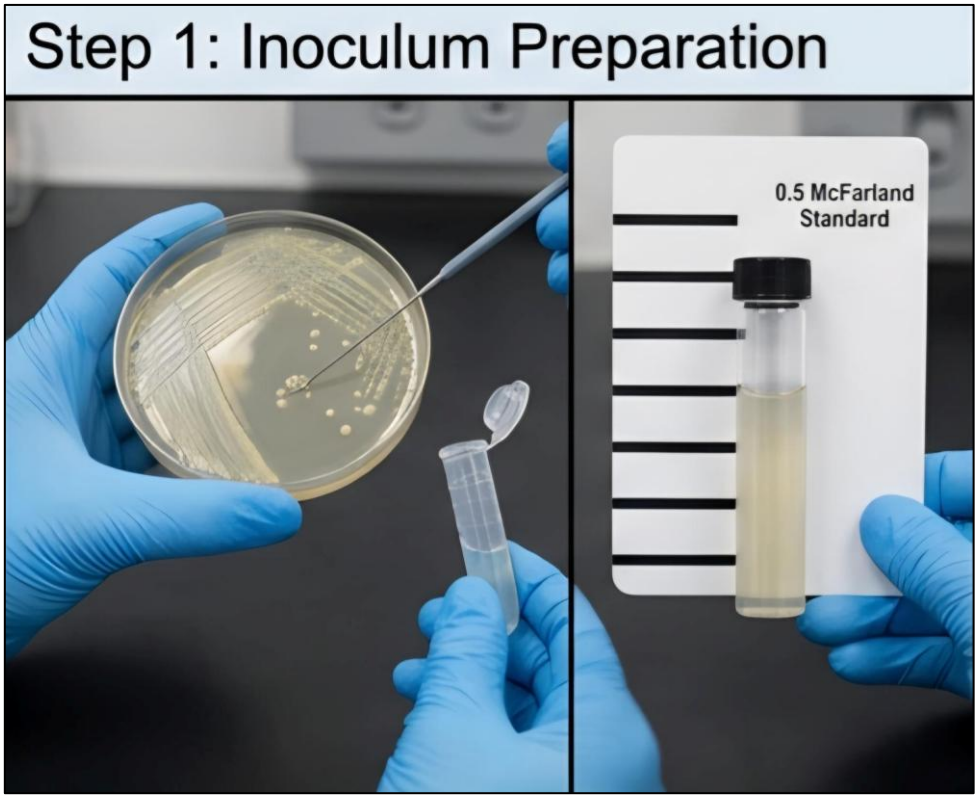
- **Antimicrobial susceptibility testing (AST)** is performed to determine the susceptibility or resistance of a bacterial isolate to specific antimicrobial agents.
- In the **Kirby–Bauer disc diffusion method**, a standardized bacterial suspension is inoculated onto the surface of **Mueller–Hinton agar**, following which antimicrobial-impregnated discs are placed on the inoculated agar surface.
- The antimicrobial agents **diffuse from the discs into the surrounding agar**. If the bacterial isolate is susceptible to the antimicrobial agent, bacterial growth is inhibited, resulting in a **clear zone of inhibition** around the disc.
- The **diameter of the zone of inhibition** is measured in millimetres and interpreted according to standardized susceptibility breakpoints provided by recognized guidelines, such as those of the **Clinical and Laboratory Standards Institute (CLSI)** or the **European Committee on Antimicrobial Susceptibility Testing (EUCAST)**.

(5) Equipment and Materials

- Bacterial culture (18–24 hour growth)
- Mueller–Hinton agar plates- quality controlled
- Sterile normal saline
- Sterile cotton swabs
- Antibiotic discs
- 0.5 McFarland turbidity standard
- Sterile forceps/disc dispenser
- Sterile test tubes
- Inoculating loop
- Wickerham turbidity standard card
- Discarding jar
- Incubator
- Vernier caliper (or a transparent ruler, a measuring scale) for measuring inhibition zones
- Personal protective equipment – gloves, masks if needed
- ATCC standard organisms

(6) Procedure

Step 1: Preparation of Bacterial Inoculum

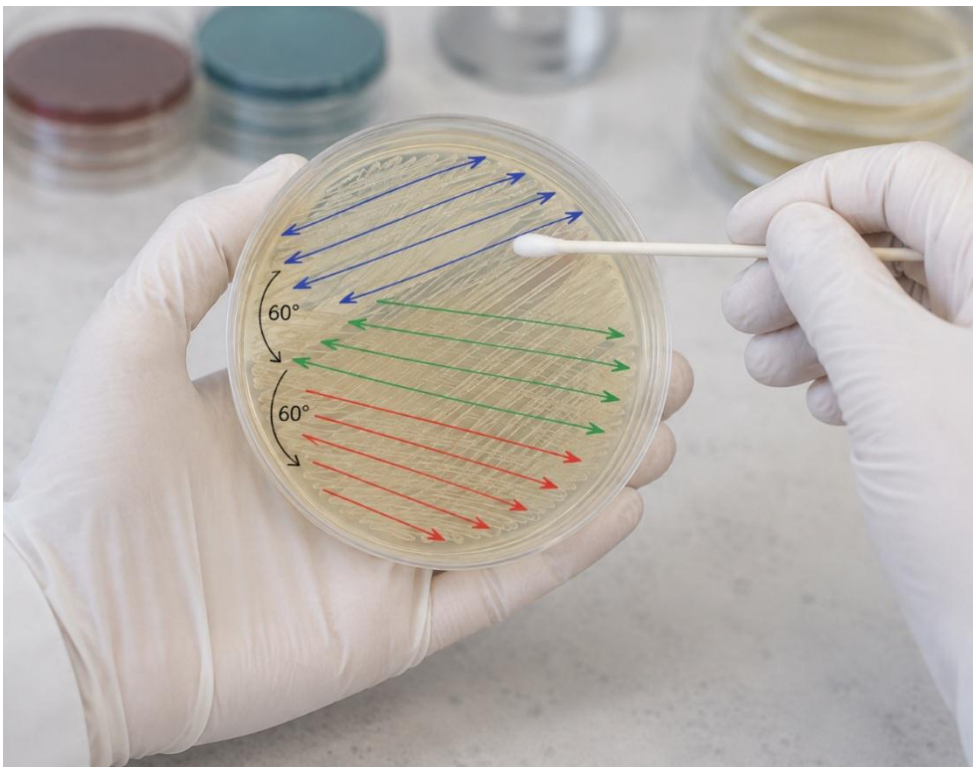


- Select 3–5 well-isolated colonies from an 18–24 hour bacterial culture using a sterile wire loop or sterile swab.
- Suspend bacterial colonies in 4-5 ml of sterile normal saline in a tube or a sterile bijou bottle.
- Adjust the turbidity of the suspension to match the 0.5 McFarland standard with the use of Wickerham turbidity standard card.
- Correct the density of test suspension to match the standard by adding more bacteria or more sterile saline.

Explanation:

- 0.5 McFarland standard turbidity of the inoculum contains approximately 1–2 × 10^8 CFU/mL of bacteria.
- Standardization of inoculum ensures a uniform number of bacterial cells are applied, allowing a confluent growth and accurate comparison of inhibition zones.

Step 2: Inoculation of Mueller–Hinton Agar



- Dip a sterile cotton swab into the standardized bacterial suspension within 15 minutes of preparation.
- Remove excess inoculum by pressing and rotating the swab against the inner wall of the tube above the level of liquid.
- Streak the swab all over the surface of Mueller–Hinton agar plate three (3) times rotating the plate through the angle of 60 ^{0}C after each application.
- Allow the plate surface to dry for a few minutes at room temperature with the lid closed.

Explanation:

- A confluent growth of bacterial lawn is required to produce accurate and reproducible inhibition zones.

Step 3: Application of Antibiotic Discs

- Place appropriate antibiotic discs onto the inoculated agar surface using a pair of sterile forceps or a disc dispenser.
- Place maximum of 5 discs on a 9 cm plate.
- Ensure equal spacing between antibiotic discs.
- Press discs gently to ensure even contact with the agar surface.
- Do not move displaced discs on the agar surface.

Explanation:

- Proper placement allows even diffusion of antimicrobial agents through the agar.

Step 4: Incubation

- Place the inoculated plates inverted in the incubator within 15 minutes of preparation.
- Incubate at 35 ± 2°C for 16–18 hours under appropriate conditions. For staphylococci and enterococci, incubate full 24 hours.

Explanation:

- Incubation allows bacterial growth and antimicrobial diffusion, producing measurable zones of inhibition.

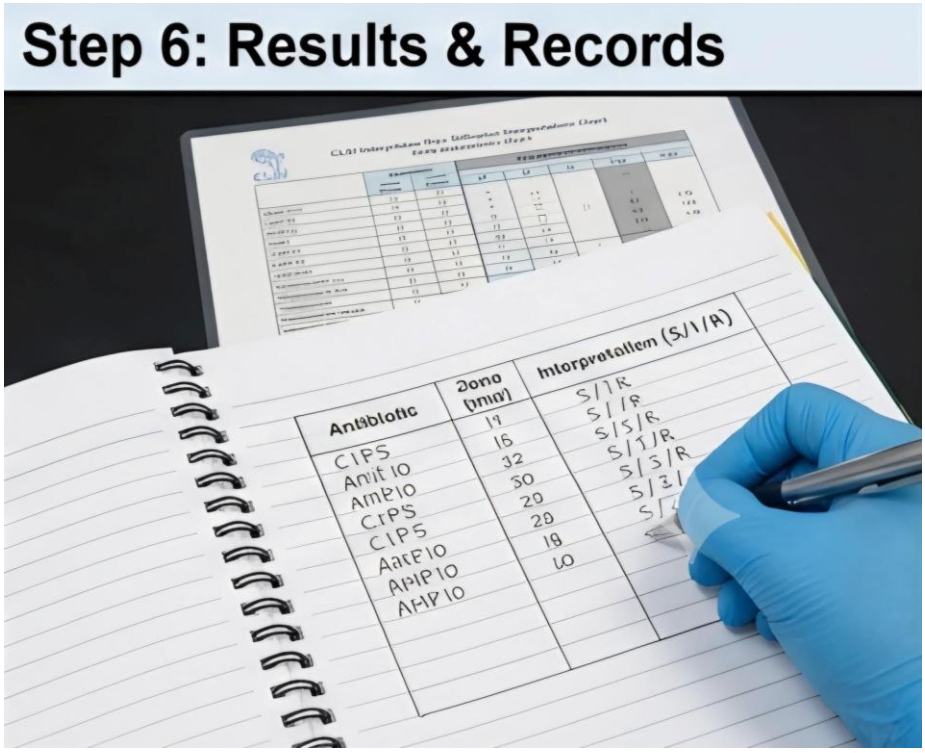
Step 5: Measurement of Inhibition Zones

- Measure the diameter of each inhibition zone (including the diameter of discs) after 16-18 hours of incubation in millimetres.
- Measure from the back of the plate using reflected light without opening the lid.
- The end point of inhibition is generally judged by the necked eye at the edge where growth starts. There are some exceptions for certain organisms and antibiotics
- Record the results.

Explanation:

- The size of the inhibition zone indicates the effectiveness of the antimicrobial agent against the tested organism.

(7) Results Interpretation



Results are interpreted according to current CLSI/EUCAST guidelines.

- Susceptible (S)
  High likelihood of successful treatment using the standard recommended dosing regimen.

- Intermediate / Susceptible Increased Exposure (I)
  Susceptible at increased exposure to the antimicrobial agent or with higher drug concentration at the site of infection.

- Resistant (R)
  High likelihood of treatment failure even when exposure to the antimicrobial is increased.

(8) Precautions

- Use a pure bacterial culture for testing
- Maintain correct inoculum density
- Use quality controlled Mueller–Hinton agar plates
- Do not use expired or improperly stored antibiotic discs
- Maintain equal spacing between antibiotic discs
- Follow aseptic techniques throughout the procedure
- Handle bacterial cultures as potentially infectious materials

(9) Post Practical Procedure

- Dispose of contaminated materials according to biomedical waste disposal guidelines
- Clean working surfaces with appropriate disinfectant
- Store unused antibiotic discs appropriately
- Switch off laboratory equipment after use
- Clean and arrange practical area

(10) Records and Documentation

- Practical number and title
- Date of experiment
- Bacterial isolate tested
- Antibiotic discs used
- Zone of inhibition measurements
- Interpretation of results
- Student name and signature
- Demonstrator verification

(11) References

- Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests. CLSI Standard M02.
- European Committee on Antimicrobial Susceptibility Testing (EUCAST). Disk Diffusion Methodology and Quality Control.
- World Health Organization (WHO). Global Antimicrobial Resistance and Use Surveillance System (GLASS).

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