Method Category	Sub-Methods	Clinical Application
Qualitative (Diffusion)	Kirby-Bauer, Stokes Method	Healthy patients with normal immunity; mild infections (e.g., uncomplicated UTIs).
Quantitative (Dilution)	Tube Dilution, Agar Dilution	Severe infections (endocarditis, osteomyelitis); high-risk/immunocompromised or critically ill patients.
Combined	**E-Test** (Epsilometer Test)	Combines diffusion and dilution principles.

4. Standardized Procedure (Kirby-Bauer)

1. **Bacterial Suspension:** Select 3-5 isolated colonies and suspend them in saline/broth.
2. **Turbidity Standard:** Adjust to **0.5 McFarland standard** (approx. 1.5×10^8 CFU/mL).
3. **Inoculation:** Use a sterile cotton swab to streak the MHA plate in three directions to create a "uniform bacterial lawn".
4. **Disk Application:** Place antibiotic-impregnated disks using sterile forceps, a template, or a dispenser.
5. **Incubation:** Incubate at $35 - 37^{\circ}C$ for 16-18 hours.
6. **Measurement:** Measure the zone diameter in millimeters (mm).

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5. Interpretation of Results

Results are interpreted using standardized charts and categorized as **Susceptible (S)**, **Intermediate (I)**, or **Resistant (R)**.

- **Using a Template:**
  - **Susceptible:** Zone edge is *outside* the black circle.
  - **Intermediate:** Zone edge lies *on* the black circle.
  - **Resistant:** No zone, or it lies *within* the white circle.

Common Antibiotic Interpretative Diameters (mm):

- **Tetracycline:** Susceptible if > 19; Resistant if < 14.
- **Erythromycin:** Susceptible if > 23; Resistant if < 13.
- **Gentamycin:** Susceptible if > 15; Resistant if < 12.

6. Quantitative Methods and The E-Test

Quantitative methods (like broth microdilution) determine the **Minimum Inhibitory Concentration (MIC)**.

- **Broth Microdilution:** Involves testing the microbe against increasing dilutions of an antimicrobial agent to find the lowest concentration that inhibits growth.

The Epsilometer Test (E-Test):

- Uses a plastic strip with a pre-defined **continuous gradient** of antibiotic.
- When placed on inoculated agar, it forms an **elliptical zone** of inhibition.
- The **MIC** is read directly from the scale where the ellipse intersects the strip.

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7. Factors Influencing Zone Size

Errors in **Inoculum Density** can lead to false results:

- **Too Light Inoculum:** Zones appear larger; resistant strains may be falsely reported as **Susceptible**.
- **Too Heavy Inoculum:** Zones appear smaller; susceptible strains may be falsely reported as **Resistant**.

Summary Overview

This lab document focuses on identifying the correct antimicrobial treatment through **Qualitative** (Kirby-Bauer) and **Quantitative** (E-test, Dilution) methods. The **Kirby-Bauer** test is the primary low-cost tool for healthy patients, while **MIC** determinations (Quantitative) are reserved for severe or high-risk cases. Success depends on strict adherence to the **0.5 McFarland standard** and the use of **Mueller-Hinton agar**.