Disk Diffusion Susceptibility Testing (Kirby-Bauer Method)

1. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility. Identification procedures may be performed at the same time. Mixtures of different types of microorganisms should not be tested on the same plate. The practice of conducting susceptibility tests directly with clinical material should be avoided. When the nature of the infection is not clear and the specimen contains mixed growth or normal flora, in which the organisms probably bear little relationship to the infection being treated, susceptibility tests are often not necessary and the results can be grossly misleading.

2. Of the many media available, NCCLS recommends Mueller-Hinton agar due to: it results in good batch-to-batch reproducibility; it is low in sulfonamide,trimethoprim, and tetracycline inhibitors; it results in satisfactory growth of most bacterial pathogens; and a large amount of data has been collected concerning susceptibility tests performed with this medium. If batches of media do not support adequate growth of organism, the zone size will be larger and provide false result. Thus, only media from manufacturers following the NCCLS standards are to be used.

3. The agar medium should have pH 7.2 to 7.4 at room temperature. The surface should be moist but without droplet of moisture. The antibiotic disks should be maintained at 8°C or lower or freeze at -14°C or below until needed, according to the manufacturer's recommendations. Allow the disks to warm to room temperature before use. Don't use expired disks.

4. To standardize the inoculum density, a BaS04 turbidity standard is used (0.5 McFarland standard,approx. 10^s organism per mL).

5. The steps of the standard method are as follows:

- a. Select at least 4 to 5 well-isolated colonies of the same morphological type from an agar plate. Touch the top of each colony with a wire loop and transfer the growth to a tube containing 4 to 5 mL of a suitable broth medium, such as tryptic-soy broth. Allow the broth culture to incubate at 35°C until it achieves or exceeds the turbidity of 0.5 McFarland standard. For routine susceptibility tests, however, the inoculum can also be prepared by making a direct saline or broth suspension of colonies that are selected from an 18 to 24-hour agar plate (a nutrient, non-selective agar such as blood agar plate must be used).

- Adjust the turbidity with sterile saline or broth. Use adequate light, and, to aid in the visual comparison, read the tube against a white background with contrasting black lines.

- Within 15 minutes after adjusting the turbidity of the inoculum suspension, dip a sterile non-toxic swab on an applicator into the adjusted suspension. Rotate the swab several times, pressing firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab.

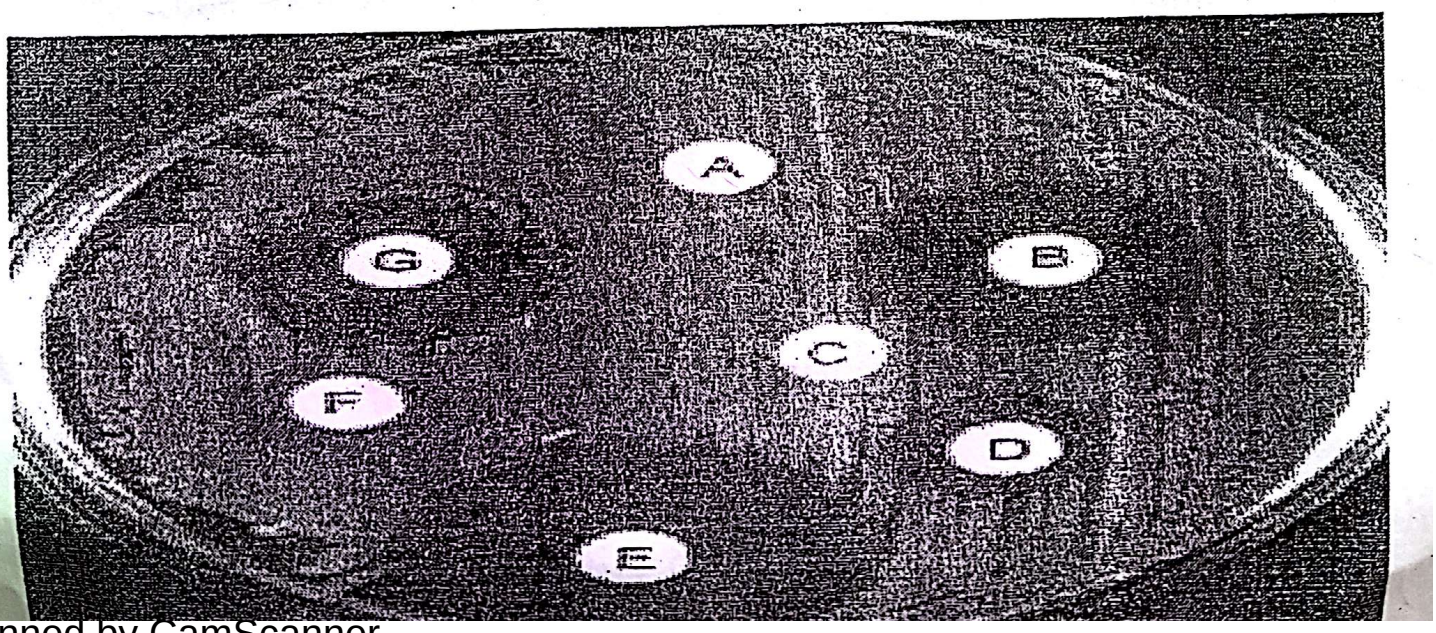
- Inoculate the dried surface of a Muller-Hintonagar plate by streaking the swab over the entire sterile agar surface. Repeat this procedure two more times, and rotate the plate 60° each time to ensure an even distribution of inoculum. Replace the plate top and allow 3 to 5 minutes, but no longer than 15 minutes, for any excess surface moisture to be absorbed before applying the antibiotic disks. There should be an almost confluent lawn of growth when done properly. If only isolated colonies grow, the inoculum was too light and the test should be repeated. To avoid extremes in inoculum density, never use undiluted overnight broth cultures for streaking plates.

- Place the appropriate disks evenly (no closer than 24 mm from center to center) on the surface of the agar plate either by using a sterile forceps or the dispensing apparatus. No more than 12 disks should be placed on one 150 mm plate or more than 5 disks on a 100 mm plate. A disk is not to be moved once it has come in contact with the agar surface since some of the compound diffuses almost instantaneously.

- Invert the plate and place them in an incubator at 35°C within 15 minutes after disks are applied. The plates should be incubated aerobically (no C02). After 16-18 hrs.of incubation, examine each plate and measure the diameters of the zones of complete inhibition, including the diameter of the disk. Measure the zones to the nearest millimeter using a ruler. Large colonies growing within a clear zone of inhibition should be subcultured, reidentified and retested./li>

- Interpret the zone sizes by referring to the manufacturer provided standard table and report the organism to be either susceptible, intermediate, or resistant. Never compare the zone sizes of two different antibiotics and judge their effectiveness accordingly.

- Quality control organisms such as E. coli ATCC 25922, and S.aureusATCC 25923 should be tested periodically to validate the accuracy of your procedures.



Exercise 5: Antibiotic Sensitivity Testing Disc)

The most useful, and certainly the most widely used, laboratory test for antimicrobial **Diffusion (Bauer-Kirby Method** susceptibility is the antimicrobial disk-agar diffusion procedure of Bauer and Kirby. There are numerous variables involve in this test; for example, selection and concentration of antimicrobial diseases; selection, volume, and age of plating medium; storage and handing of discs; and number of bacterial cells, and international criteria used for interpreting the result.

Materials – for demonstration

1. Mueller-Hinton Broth tube with recommended number for bacteria (10^6 cells/ml).
2. Muller-Hinton agar plate.
3. Sterile cotton swab.
4. Antimicrobial discs.
5. 0.5 Macfarland standard.

Procedure

(First Period)

a) Dip sterile swab into the standardized culture and streak the plate for confluent growth by streaking the entire plate, rotating the plate 90° restreaking a third time after again rotating the plate.

b) Permit plate to dry for approximately 3-5 min before applying the set of discs. Tamp discs down gently on to the agar using a flamed and cooled forceps. Incubate plates inverted.

(Second Period)

a) Measure the diameter of the zone of inhibition using the mm rulers. Record the zones for each antibiotic disc to the closest whole number.

b) Read of the sensitivity chart for each antibiotic whether the organism is sensitive or resistant. For demonstration see the prepared sensitivity plates.

...meter Interpretive Standards and Approximate Minimum Inhibitory Concentration (MIC) Correlates

Antimicrobial Agent	Disk Content	Zone Diameter, nearest whole mm				Approximate MIC Correlates[a]	
		Resistant	Intermediate[b]	Moderately Susceptible[b]	Susceptible	Resistant	Susceptible
Amikacin[c]	30µg	≤ 14	15–16	—	≥ 17	≥ 32µg/mL	≤ 16µg/mL
Ampicillin[d] when testing gram-negative enteric organisms	10µg	≤ 11	12–13	—	≥ 14	≥ 32µg/mL	≤ 8µg/mL
when testing staphylococci[e]	10µg	≤ 28	—	—	≥ 29	β-lactamase[e]	≤ 0.25µg/mL
when testing *Haemophilus* species[f]	10µg	≤ 19	—	—	≥ 20	≥ 4µg/mL	≤ 2µg/mL
when testing enterococci[g,h]	10µg	≤ 16	—	≥ 17[h]	—	≥ 16µg/mL	—
when testing non-enterococcal streptococci and *Listeria monocytogenes*[g,h]	10µg	≤ 21	—	22–29	≥ 30	≥ 4µg/mL	≤ 0.12µg/mL
Augmentin when testing *Haemophilus* & staphylococci[e,i]	20/10µg	≤ 19	—	—	≥ 20	—	≤ 4/2µg/mL
when testing other organisms	20/10µg	≤ 13	14–17	—	≥ 18	≥ 32/16µg/mL	≤ 8/4µg/mL
Aztreonam when testing *Pseudomonas*[e]	75µg	≤ 14	15–17	—	≥ 18	≥ 256µg/mL	≤ 64µg/mL
Carbenicillin when testing the Enterobacteriaceae[e]	30µg	≤ 15	—	16–21	≥ 22	≥ 32µg/mL	≤ 8µg/mL
when testing *Pseudomonas*	100µg	≤ 17	18–22	—	≥ 23	≥ 32µg/mL	≤ 16µg/mL
Cefamandole[j]	100µg	≤ 13	14–16	—	≥ 17	≥ 512µg/mL	≤ 128µg/mL
Cefazolin[j] (KZ) 2.G.	30µg	≤ 14	15–17	—	≥ 18	≥ 32µg/mL	≤ 8µg/mL
Cefonicid[j] 2.G.	30µg	≤ 14	15–17	—	≥ 18	≥ 32µg/mL	≤ 8µg/mL
Cefoperazone[j] 3.G. Cph.	30µg	≤ 14	15–17	—	≥ 18	≥ 32µg/mL	≤ 8µg/mL
Cefotaxime[j] 3.G. cephalosporin	75µg	≤ 15	—	16–20	≥ 21	≥ 64µg/mL	≤ 16µg/mL
Cefoxitin[j] 2.G "	30µg	≤ 14	—	15–22	≥ 23	≥ 64µg/mL	≤ 8µg/mL
Ceftazidime[j]	30µg	≤ 14	15–17	—	≥ 18	≥ 32µg/mL	≤ 8µg/mL
Ceftizoxime when testing urinary isolates of *P. aeruginosa*	30µg	≤ 14	15–17	—	≥ 18	≥ 32µg/mL	≤ 8µg/mL
when testing other organisms	30µg	≤ 10	—	≥ 11	—	≥ 64µg/mL	—
Ceftriaxone (ZOX) 3.G.	30µg	≤ 14	—	15–19	≥ 20	≥ 32µg/mL	≤ 8µg/mL
Cefuroxime[j] (CXM) 2.G	30µg	≤ 13	—	14–20	≥ 21	≥ 64µg/mL	≤ 8µg/mL
Cephalothin[j] (KF) 1.G	30µg	≤ 14	15–17	—	≥ 18	≥ 32µg/mL	≤ 8µg/mL
Chloramphenicol	30µg	≤ 14	15–17	—	≥ 18	≥ 32µg/mL	≤ 8µg/mL
Cinoxacin[k]	30µg	≤ 12	13–17	—	≥ 18	≥ 25µg/mL	≤ 12.5µg/mL
Clindamycin[l]	100µg	≤ 14	15–18	—	≥ 19	≥ 64µg/mL	≤ 16µg/mL
Doxycycline[m]	2µg	≤ 14	15–16	—	≥ 17	≥ 2µg/mL	≤ 1µg/mL
Erythromycin	30µg	≤ 12	13–15	—	≥ 16	≥ 16µg/mL	≤ 4µg/mL
Gentamicin[c] (CN) (G)	15µg	≤ 13	14–17	—	≥ 18	≥ 8µg/mL	≤ 2µg/mL
Imipenem	10µg	≤ 12	13–14	—	≥ 15	≥ 8µg/mL	≤ 4µg/mL
Kanamycin	10µg	≤ 13	14–15	—	≥ 16	≥ 16µg/mL	≤ 4µg/mL
Methicillin when testing staphylococci[e]	30µg	≤ 13	14–17	—	≥ 18	≥ 25µg/mL	≤ 6µg/mL
Mezlocillin[e]	5µg	≤ 9	10–13	—	≥ 14	—	≤ 3µg/mL
Minocycline[m]	75µg	≤ 12	13–15	—	≥ 16	≥ 256µg/mL	≤ 64µg/mL
Moxalactam[j]	30µg	≤ 14	15–18	—	≥ 19	≥ 16µg/mL	≤ 4µg/mL
Nafcillin when testing staphylococci[n]	30µg	≤ 14	—	15–22	≥ 23	≥ 64µg/mL	≤ 8µg/mL
Nalidixic Acid[k] (NA)	1µg	≤ 10	11–12	—	≥ 13	—	≤ 1µg/mL
Netilmicin[c]	30µg	≤ 13	14–18	—	≥ 19	≥ 32µg/mL	≤ 8µg/mL
Nitrofurantoin[k] (F)	30µg	≤ 12	13–14	—	≥ 15	≥ 32µg/mL	≤ 12µg/mL
Norfloxacin (NOR)	300µg	≤ 14	15–16	—	≥ 17	≥ 100µg/mL	≤ 25µg/mL
Oxacillin when testing staphylococci[n]	10µg	≤ 12	13–16	—	≥ 17	≥ 16µg/mL	≤ 4µg/mL
when testing pneumococci for penicillin G susceptibility	1µg	≤ 10	11–12	—	≥ 13	—	≤ 1µg/mL
Penicillin G when testing staphylococci[e]	1µg	≤ 19	—	—	≥ 20	—	≤ 0.06µg/mL
when testing *N. gonorrhoeae*	10 units	≤ 28	—	—	≥ 29	β-lactamase[e]	≤ 0.1µg/mL
when testing enterococci[g,h]	10 units	≤ 19	—	—	≥ 20	β-lactamase	≤ 0.1µg/mL
when testing non-enterococcal streptococci and *L. monocytogenes*[g,h]	10 units	≤ 14	—	≥ 15[h]	—	≥ 16µg/mL	—
Piperacillin[e]	100µg	≤ 19	—	20–27	≥ 28	≥ 4µg/mL	≤ 0.12µg/mL
Streptomycin	10µg	≤ 14	15–17	—	≥ 18	≥ 256µg/mL	≤ 64µg/mL
Sulfonamides[k,o]	250 or 300 µg	≤ 11	12–14	—	≥ 15	—	—
Tetracycline[m] (TE)	30µg	≤ 12	13–16	—	≥ 17	≥ 350µg/mL	≤ 100µg/mL
Ticarcillin[e]	75µg	≤ 14	15–18	—	≥ 19	≥ 16µg/mL	≤ 4µg/mL
Tobramycin[c]	10µg	≤ 11	12–14	—	≥ 15	≥ 128µg/mL	≤ 64µg/mL
Trimethoprim[l,o]	5µg	≤ 12	13–14	—	≥ 15	≥ 8µg/mL	≤ 4µg/mL
Trimethoprim-sulfamethoxazole[o] (SXT)	1.25/23.75µg	≤ 10	11–15	—	≥ 16	≥ 16µg/mL	≤ 4µg/mL
Vancomycin	30µg	≤ 9	10–11	—	≥ 12	≥ 8/152µg/mL	≤ 2/38µg/mL
						—	≤ 5µg/mL