Kirby-Bauer Disk Diffusion Susceptibility Test Protocol

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Information

History

The publication on penicillin by Alexander Fleming in 1928 is a milestone in the history of medicine. As more antimicrobial compounds were discovered, it was predicted that infectious diseases would be eliminated through the use of these antimicrobials (7). Unfortunately, the development of bacterial resistance to these antimicrobials quickly diminished this optimism and resulted in the need for physicians to request the microbiology lab to test a patient's pathogen against various concentrations of a given antimicrobial to determine susceptibility or resistance to that drug. The original method of determining susceptibility to antimicrobials was based on broth dilution methods (5, 7), which although still the gold standard today, are time consuming to perform. This prompted the development of a disk diffusion procedure for the determination of susceptibility of bacteria to antimicrobials.

By the early 1950s, most clinical microbiology laboratories in the United States had adopted the disk diffusion method for determining susceptibility of bacteria to antimicrobials. Each lab modified the procedure to suit its own needs, which included using different types of media, inoculum concentration, incubation time, incubation temperature, and concentration of the antimicrobial compound. Interpretation of susceptibility and resistance was based only on the presence or absence of a zone of inhibition surrounding the disk, and two or three different concentrations of the same antimicrobial were routinely tested against the pathogen (1). Many researchers published variations for the procedure resulting in multiple protocols that resulted in widespread confusion (1). In 1956, W. M. M. Kirby and his colleagues at the University of Washington School of Medicine and the King County Hospital proposed a single disk method for antimicrobial susceptibility testing (6).

The lack of standardization for the determination of bacterial susceptibility continued to be a problem throughout the early 1960s. Kirby and his colleague, A. W. Bauer, extensively reviewed the susceptibility testing literature. They consolidated and updated all the previous descriptions of the disk diffusion method and published their findings (2). This publication led the World Health Organization to form a committee in 1961 to lay the groundwork for the development of a standardized procedure for single antimicrobial disk susceptibility testing (7). The result was a standardized procedure for the disk diffusion

susceptibility test, henceforth called the Kirby-Bauer disk diffusion test (2).

Currently, the Clinical Laboratory Standards Institute (CLSI) is responsible for updating and modifying the original procedure of Kirby and Bauer through a global consensus process. This ensures uniformity of technique and reproducibility of results as pathogens develop new mechanisms of resistance and new antimicrobials are developed to fight these organisms. Interpretative guidelines for zone sizes are included in their publications (3). The CLSI publication, Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard 9th Edition, represents the standard for clinical laboratories performing susceptibility testing today.

Purpose

The purpose of the Kirby-Bauer disk diffusion susceptibility test is to determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria to various antimicrobial compounds in order to assist a physician in selecting treatment options for his or her patients. The pathogenic organism is grown on Mueller-Hinton agar in the presence of various antimicrobial impregnated filter paper disks. The presence or absence of growth around the disks is an indirect measure of the ability of that compound to inhibit that organism.

Theory

Determination of bacterial resistance to antimicrobials is an important part of the management of infections in patients. The disk diffusion method of Kirby and Bauer has been standardized and is a viable alternative to broth dilution methods for laboratories without the resources to utilize the newer automated methods for broth microdilution testing.

When a 6-mm filter paper disk impregnated with a known concentration of an antimicrobial compound is placed on a Mueller-Hinton (MH) agar plate, immediately water is absorbed into the disk from the agar. The antimicrobial begins to diffuse into the surrounding agar. The rate of diffusion through the agar is not as rapid as the rate of extraction of the antimicrobial out of the disk, therefore the concentration of antimicrobial is highest closest to the disk and a logarithmic reduction in concentration occurs as the distance from the disk increases (7). The rate of diffusion of the antimicrobial through the agar is dependent on the diffusion and solubility properties of the drug in MH agar (2) and the molecular weight of the antimicrobial compound. Larger molecules will diffuse at a slower rate than lower molecular weight compounds. These factors, in combination, result in each antimicrobial having a unique breakpoint zone size indicating susceptibility to that antimicrobial compound.

If the agar plate has been inoculated with a suspension of the pathogen to be tested prior to the placing of disks on the agar surface, simultaneous growth of the bacteria and diffusion of the antimicrobial compounds occurs. Growth occurs in the presence of an antimicrobial

compound when the bacteria reach a critical mass and can overpower the inhibitory effects of the antimicrobial compound. The estimated time of a bacterial suspension to reach critical mass is 4 to 10 hours for most commonly recovered pathogens, but is characteristic of each species, and influenced by the media and incubation temperature (7). The size of the zone of inhibition of growth is influenced by the depth of the agar, since the antimicrobial diffuses in three dimensions, thus a shallow layer of agar will produce a larger zone of inhibition than a deeper layer.

The point at which critical mass is reached is demonstrated by a sharply marginated circle of bacterial growth around the disk. The concentration of antimicrobial compound at this margin is called the critical concentration and is approximately equal to the minimum inhibitory concentration obtained in broth dilution susceptibility tests.

Zone size observed in a disk diffusion test has no meaning in and of itself (7). The interpretation of resistance and susceptibility to antimicrobials is determined through in vivo testing of blood and urine to calculate the obtainable level of a given antimicrobial that results in resolution of an infection. This information is correlated with zone sizes resulting in the interpretive standards. The current interpretation standards can be found in the Clinical Laboratory Standards Institute Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standards 9th Edition (3).

RECIPE

Sterile saline in 2-ml tubes	18- to 24-ho
0.5 McFarland standard	Vortex
Wickerham card	Sterile swabs
Mueller-Hinton agar plates, 100 mm or 150 mm	Inoculating l
Caliper or ruler	Bact-cinerat
Antibiotic disks[b]	Alcohol pads
Forceps	35°C to 37°C
Antibiotic disk dispenser (optional)	

[a]Recommended organisms for quality assurance purposes are Staphylococcus aureus ATCC 25923 (Biosafety level (BSL) 2), Escherichia coli ATCC 25922 (BSL 1), and Pseudomonas aeruginosa ATCC 27853 (BSL 2) (www.atcc.org), as the zone of inhibition for these organisms is known. Because the zone sizes are known for these organisms, they are recommended for use in the educational setting, although the use of unknowns should also be incorporated into the educational experience. For quality control testing, the zone sizes for these three organisms can be found on the package insert from any antimicrobial disk you purchase.

[b]Selection of antimicrobial is based on the type of organism being tested and source of the isolate (blood, urine, wound, etc.). See the Interpretative Standards Tables for suggested antimicrobials to use in this exercise.

Additional Notes

Mueller-Hinton agar_

MH agar is considered the best medium to use for routine susceptibility testing of nonfastidious bacteria for the following reasons:

· It shows acceptable batch-to-batch reproducibility for susceptibility testing
· It is low in sulfonamide, trimethoprim, and tetracycline inhibitors
· It supports satisfactory growth of most nonfastidious pathogens
· A large body of data and experience has been collected concerning susceptibility tests performed with this medium (6).

Please note that the use of media other than Mueller-Hinton agar may result in erroneous results. Also note that only the aerobic or facultative bacteria that grow well on unsupplemented MH agar should be tested using this protocol. Fastidious organisms require MH agar supplemented with additional nutrients and require that modification to this protocol be made. Neither the supplements nor the procedural modification are discussed in this basic protocol.

MH agar may be purchased as prepared agar plates from Remel (Lenexa, KS), BD BBL (Franklin Lakes, NJ), or any other supplier of prepared agar plates. Follow the manufacturer's recommendation for storage of prepared plates. MH agar can also be prepared from dehydrated media available from companies such as Remel, BD BBL, or any other supplier of dehydrated media. Be sure to prepare the media according to the manufacturer's directions.

Formula for Mueller-Hinton agar per liter of purified water (4)

Beef, Infusion from	300.0 g
Casamino acid, technical	17.5 g
Starch	1.5 g
Agar	17.0 g

Suspend the components listed above in 1 liter of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the components. Autoclave at 121°C for 15 minutes. Dispense as desired. Allow to solidify at room temperature, then store at 4 to 8°C. Mueller-Hinton agar is stable for approximately 70 days (per Remel Technical Services, 1 September 2009) from the date of preparation. Each lab should verify the quality and functionality of each batch of prepared media by testing known strains of organisms against each antimicrobial compound being used as the 70-day expiration date approaches.

· If you prepare the MH agar plates from dehydrated media, the plates must be poured to a depth of 4 mm (approximately 25 ml of liquid agar for 100-mm plates and 60 ml of liquid agar for 150-mm plates, but in any case to a measured depth of 4 mm). Plates that are too shallow will produce false susceptible results as the antimicrobial compound will diffuse further than it should, creating larger zones of

inhibition. Conversely, plates poured to a depth >4 mm will result in false resistant results.

· pH of the MH agar should fall between 7.2 and 7.4 at room temperature after solidification and should be tested when the media is first prepared. If the pH is <7.2 certain drugs will appear to lose potency (aminoglycosides, quinolones, macrolides), while other agents may appear to have excessive activity (tetracycline). If the pH is >7.4, the opposite results may occur.

· Excessive thymidine or thymine can reverse the inhibitory effects of sulfonamides and trimethoprim resulting in smaller and less distinct zones of inhibition, or no zones at all.

· The incorrect concentration of divalent cations (calcium and magnesium) will affect the results of aminoglycoside and tetracycline tests against Pseudomonas aeruginosa. Excess cation concentration will result in reduced zone sizes and low concentration will increase zone sizes. Excess calcium will increase the zone size of P. aeruginosa against daptomycin. Excess zinc ions may reduce the zone size of carbapenems against P. aeruginosa.

· MH agar should be tested with known strains of organism at least weekly in order to verify that the media and disks are working as expected.

Antibiotic susceptibility disks

Antimicrobial disks can be purchased from any reputable suppliers, such as Remel or BD BBL. They are packaged in spring-loaded cartridges containing 25 or 50 disks and can be ordered as individual cartridges or in packages of 10 cartridges. Proper storage of these disks is essential for reproducible results.

Sealed cartridges containing commercially prepared paper disks should be stored at either 8°C or frozen at -14°C in a non-self-defrosting freezer. Allow disks to come to room temperature prior to removing the protective plastic packaging. Once opened, store the cartridges in a storage container containing desiccant for no more than 1 week.

Semiautomatic disk dispensers are available from companies such as Remel and BD BBL. Be aware that disk cartridges from one company may not fit the dispenser of another company.

McFarland standard

McFarland standards are suspensions of either barium sulfate or latex particles that allow visual comparison of bacterial density (Fig. 1). Commercially prepared standards are available for purchase from companies such as Remel or BD BBL. These often include a Wickerham card, which is a small card containing parallel black lines. A 0.5 McFarland standard is equivalent to a bacterial suspension containing between 1 x 108 and 2 x 108 CFU/ml of E. coli.

A 0.5 McFarland standard may be prepared in-house as describe below.

1. Add a 0.5-ml aliquot of a 0.048 mol/liter $BaCl_2$ (1.175% wt/vol $BaCl_2 \cdot 2H_2O$) to 99.5 ml of 0.18 mol/liter H_2SO_4 (1% vol/vol) with constant stirring to maintain a suspension.

2. Verify the correct density of the turbidity standard by measuring absorbance using a spectrophotometer with a 1-cm light path and matched cuvette. The absorbance at 625 nm should be 0.08 to 0.13 for the 0.5 McFarland standard.

3. Transfer the barium sulfate suspension in 4- to 6-ml aliquots into screw-cap tubes of the same size as those used in standardizing the bacterial inoculums.

4. Tightly seal the tubes and store in the dark at room temperature.

Use of the McFarland standard in the Kirby-Bauer procedure.

1. Prior to use, vigorously agitate the barium sulfate standard on a mechanical vortex mixer and inspect for a uniformly turbid appearance. Replace the standard if large particles appear. If using a standard composed of latex particles, mix by inverting gently, not on a vortex mixer.

2. As the student adds bacterial colonies to the saline in the "preparation of the inoculum" step of the procedure, he or she should compare the resulting suspension to the McFarland standard. This is done by holding both the standard and the inoculum tube side by side and no more than 1 inch from the face of the Wickerham card (with adequate light present) and comparing the appearance of the lines through both suspensions. Do not hold the tubes flush against the card. If the bacterial suspension appears lighter than the 0.5 McFarland standard, more organisms should be added to the tube from the culture plate. If the suspension appears more dense than the 0.5 McFarland standard, additional saline should be added to the inoculum tube in order to dilute the suspension to the appropriate density. In some cases it may be easier to start over rather than to continue to dilute a bacterial suspension that is too dense for use.

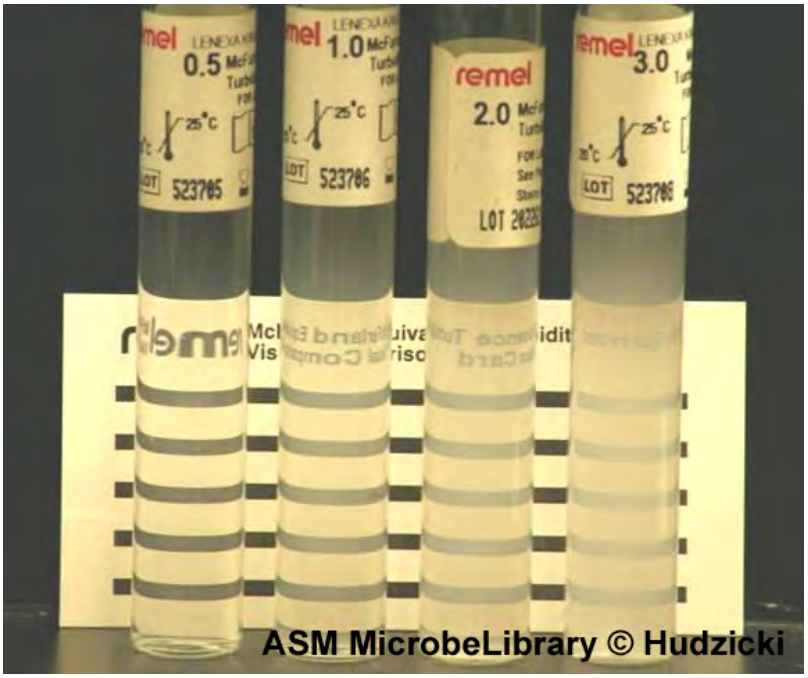


FIG. 1. McFarland standards (left to right) 0.5, 1.0, 2.0, 3.0, positioned in front of a Wickerham card. McFarland standards are used to prepare bacterial suspensions to a specified turbidity. In the Kirby-Bauer disk diffusion susceptibility test protocol, the bacterial suspension of the organism to be tested should be equivalent to the 0.5 McFarland standard.

PROTOCOL

Preparation of Mueller-Hinton plate

1. Allow a MH agar plate (one for each organism to be tested) to come to room temperature. It is preferable to allow the plates to remain in the plastic sleeve while they warm to minimize condensation.

2. If the surface of the agar has visible liquid present, set the plate inverted, ajar on its lid to allow the excess liquid to drain from the agar surface and evaporate. Plates may be placed in a 35°C incubator or in a laminar flow hood at room temperature until dry (usually 10 to 30 minutes).

3. Appropriately label each MH agar plate for each organism to be tested.

Preparation of inoculum

1. Using a sterile inoculating loop or needle, touch four or five isolated colonies of the organism to be tested.

2. Suspend the organism in 2 ml of sterile saline.

3. Vortex the saline tube to create a smooth suspension.

4. Adjust the turbidity of this suspension to a 0.5 McFarland standard by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy.

5. Use this suspension within 15 minutes of preparation.

Additional Notes

Inoculum preparation

Organisms to be tested must be in the log phase of growth in order for results to be valid. It is recommended that subcultures of the organisms to be tested be made the previous day.

Never use extremes in inoculum density. Never use undiluted overnight broth cultures or other unstandardized inocula for inoculating plates.

If the organism is difficult to suspend directly into a smooth suspension, the growth method of preparing the inoculums should be used. However, the recommended organisms listed in this procedure all produce smooth suspensions with little difficulty. See the Clincial Laboratory Standards Institute document (3) for the growth procedure method for preparing the inoculums, if needed.

Inoculation of the MH plate

1. Dip a sterile swab into the inoculum tube.

2. Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. The swab should not be dripping wet (Fig. 2).

3. Inoculate the dried surface of a MH agar plate by streaking the swab three times over the entire agar surface; rotate the plate approximately 60 degrees each time to ensure an even distribution of the inoculum (Fig. 3).

4. Rim the plate with the swab to pick up any excess liquid (Fig. 4).

5. Discard the swab into an appropriate container.

6. Leaving the lid slightly ajar, allow the plate to sit at room temperature at least 3 to 5 minutes, but no more than 15 minutes, for the surface of the agar plate to dry before proceeding to the next step.

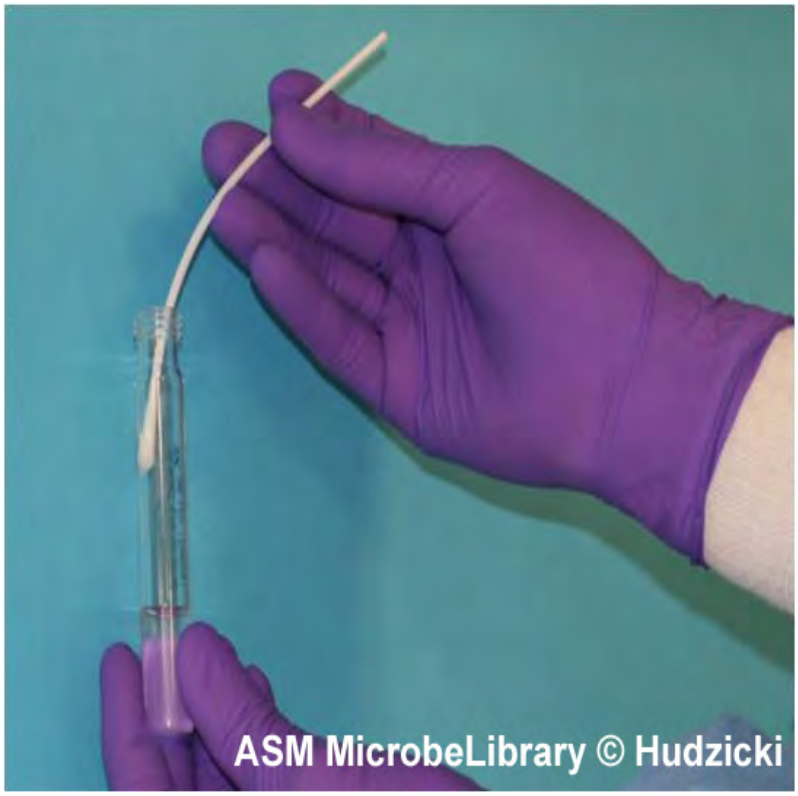
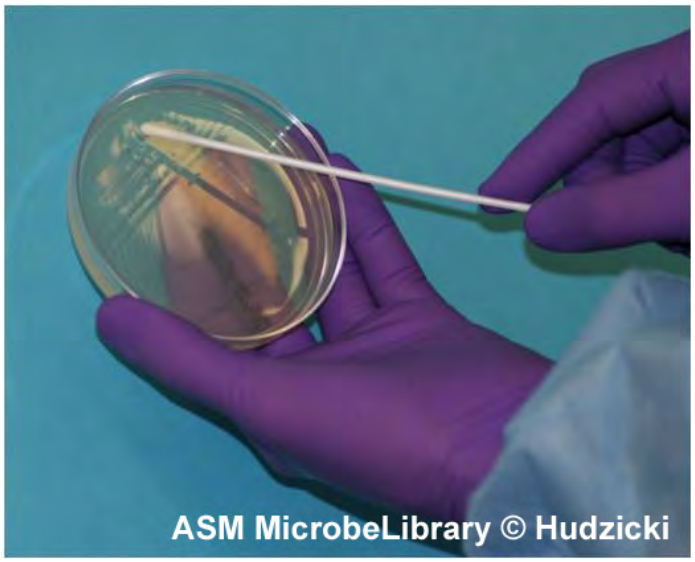
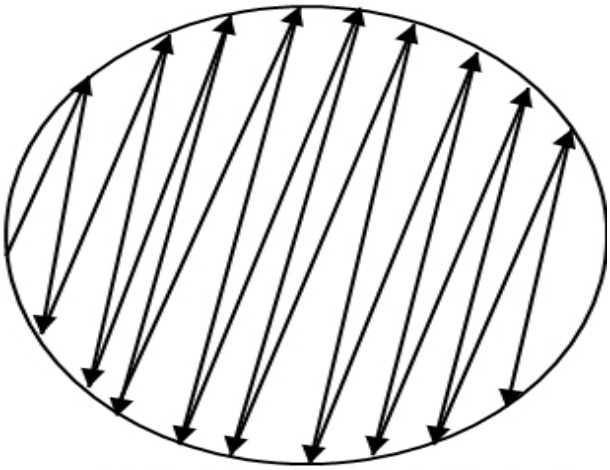


FIG. 2. Kirby-Bauer disk diffusion susceptibility test protocol, inoculation of the test plate. Step 2. Rotate the swab against the side of the tube while applying pressure to remove excess liquid from the swab prior to inoculating the plate.



A



B

FIG. 3. Kirby-Bauer disk diffusion susceptibility test protocol, inoculation of the Mueller-Hinton agar plate. Step 3. (A) Inoculate the plate with the test organism by streaking the swab in a back-and-forth motion very close together as you move across and down the plate. Rotate the plate 60° and repeat this action. Rotate the plate once more and repeat the streaking action. This ensures an even distribution of inoculum that will result in a confluent lawn of growth. (B) Diagram illustrating the pattern the swab should follow as it is drawn across the plate._

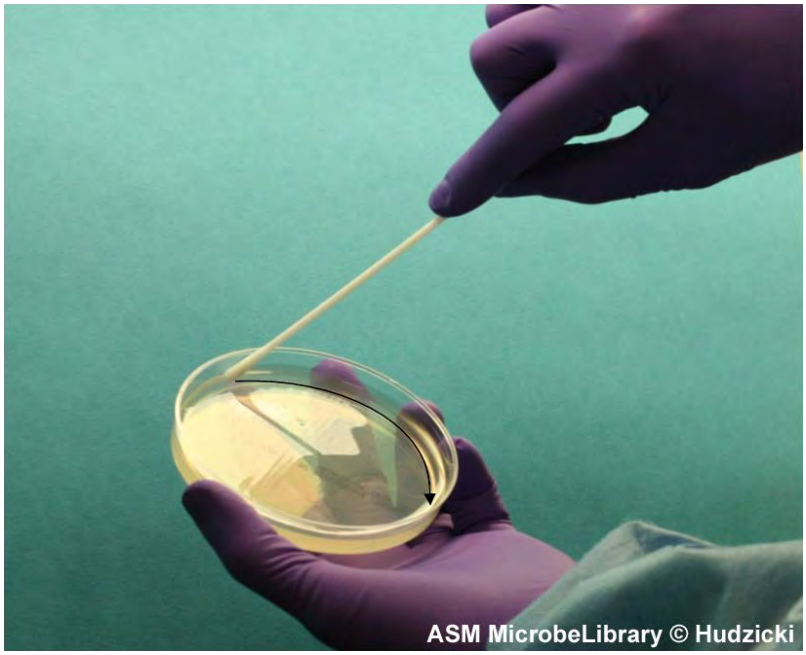


FIG. 4. Kirby-Bauer disk diffusion susceptibility test protocol, inoculation of the Mueller-Hinton agar plate. Step 4. After streaking the Mueller-Hinton agar plate as described in Step 3, rim the plate with the swab by running the swab around the edge of the entire the plate to pick up any excessive inoculum that may have been splashed near the edge. The

arrow indicates the path of the swab.

Placement of the antibiotic disks

1. Place the appropriate antimicrobial-impregnated disks on the surface of the agar, using either forceps to dispense each antimicrobial disk one at a time, or a multidisk dispenser to dispense multiple disks at one time. (See steps a. through d. for the use of the multi-disk dispenser or steps e. through g. for individual disk placement with forceps.

a. To use a multidisk dispenser, place the inoculated MH agar plate on a flat surface and remove the lid (Fig. 5).

b. Place the dispenser over the agar plate and firmly press the plunger once to dispense the disks onto the surface of the plate.

c. Lift the dispenser off the plate and using forceps sterilized by either cleaning them with an alcohol pad or flaming them with isopropyl alcohol, touch each disk on the plate to ensure complete contact with the agar surface. This should be done before replacing the petri dish lid as static electricity may cause the disks to relocate themselves on the agar surface or adhere to the lid (Fig. 6d).

d. Do not move a disk once it has contacted the agar surface even if the disk is not in the proper location, because some of the drug begins to diffuse immediately upon contact with the agar.

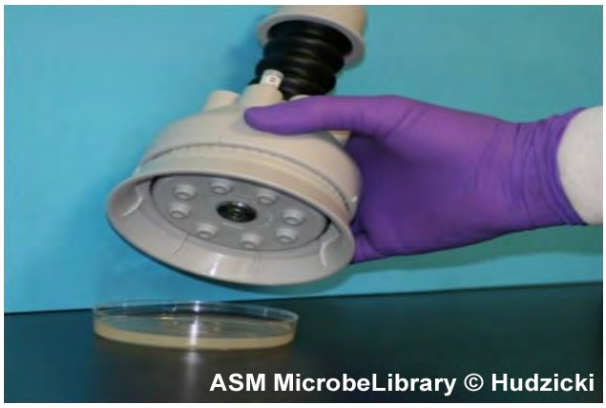
e. To add disks one at a time to the agar plate using forceps, place the MH plate on the template (Fig. 7) provided in this procedure (Fig. 6a). Sterilize the forceps by cleaning them with a sterile alcohol pad and allowing them to air dry or immersing the forceps in alcohol then igniting.

f. Using the forceps carefully remove one disk from the cartridge (Fig. 6b).

g. Partially remove the lid of the petri dish. Place the disk on the plate over one of the dark spots on the template and gently press the disk with the forceps to ensure complete contact with the agar surface. Replace the lid to minimize exposure of the agar surface to room air (Fig. 6c, d).

h. Continue to place one disk at a time onto the agar surface until all disks have been placed as directed in steps f. and g. above.

2. Once all disks are in place, replace the lid, invert the plates, and place them in a 35°C air incubator for 16 to 18 hours. When testing Staphylococcus against oxacillin or vancomycin, or Enterococcus against vancomycin, incubate for a full 24 hours before reading.



A

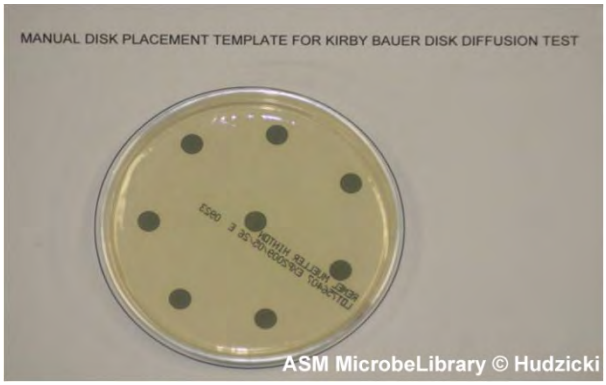


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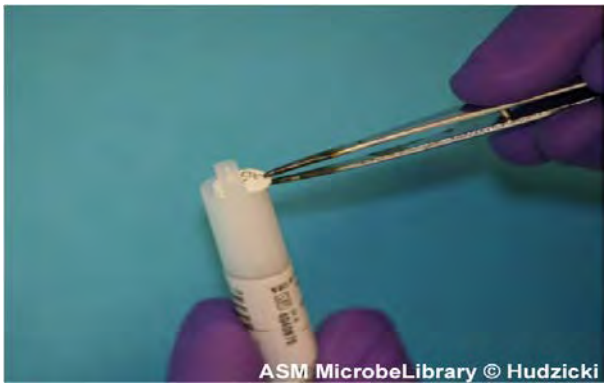


C

FIG. 5. Kirby-Bauer disk diffusion susceptibility test protocol, placement of antibiotic disks using an automated disk dispenser. Step 1, a. through d. An automatic disk dispenser can be used to place multiple disks simultaneously on a MH agar plate. () Set the dispenser over the plate. (B) Place the palm of your hand on the top of the handle. (C) Press down firmly and completely to dispense the disks. The spring loaded handle will return to the original position when pressure is removed.


MANUAL DISK PLACEMENT TEMPLATE FOR KIRBY BAUER DISK DIFFUSION TEST
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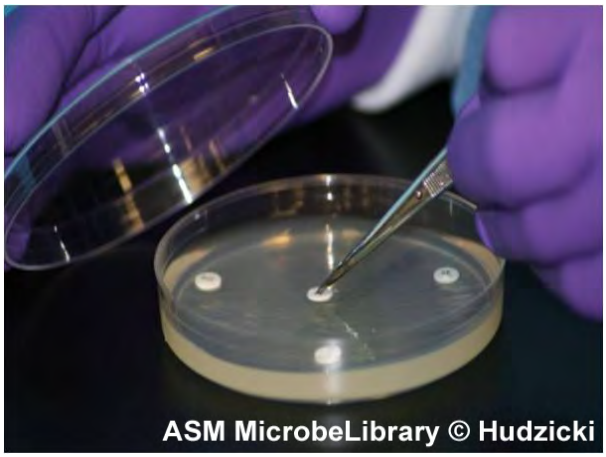
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C



D

FIG. 6. Kirby-Bauer disk diffusion susceptibility test protocol, placement of antibiotic disks using forceps to manually place the disks. Step 1, e. through h. Antibiotic disks can be manually placed on the MH agar plate if desired. (A) Place the Mueller-Hinton agar plate over the disk template. (B) Remove one disk from the cartridge using forceps that have been sterilized. (C) Lift the lid of the plate and place the disk over one of the positioning marks. (D) Press the disk with the forceps to ensure complete contact with the agar surface. Replace the lid of the plate between disks to minimize exposure to air-borne contaminants.

Additional Notes

Disk placement

Disks should not be placed closer than 24 mm (center to center) on the MH agar plate. Ordinarily, no more than 12 disks should be placed on a 150-mm plate or more than 5 disks on a 100-mm plate. However, the semiautomatic disk dispensers hold 16 and 8 disks respectively and may not maintain the recommended 24 mm center to center spacing. The template provided in this protocol (Fig. 7) maintains the recommended 24 mm center to center spacing and allows the placement of up to 8 disks on the plate.

You should avoid placing disks close to the edge of the plate as the zones will not be fully round and can be difficult to measure.

Each disk must be pressed down with forceps to ensure complete contact with the agar surface or irregular zone shapes may occur.

If the surface of the agar is disrupted in any way (a disk penetrating the surface, visible lines present due to excessive pressure of the swab against the plate during inoculation, etc.) the shape of the zone may be affected.

When printing the template for use in your microbiology lab, be sure that the diameter of the circle on the template is the same size as the Mueller-Hinton agar plates that you use in lab (100 mm). The "reduce" or

"enlarge" function on a photocopier can be used to change the size of the template if needed. You may also make your own template by drawing a circle around a MH agar plate on a sheet of paper. Add the placement marks based on the number of disks you plan to use in your lab session, maintaining the recommended spacing as indicated above.

Incubation of the plates

A temperature range of 35°C ± 2°C is required.

Note that temperatures above 35°C may not allow the detection of methicillin-resistant Staphylococcus.

Do not incubate plates in CO2 as this will decrease the pH of the agar and result in errors due to incorrect pH of the media.

Results can be read after 18 hours of incubation unless you are testing Staphylococcus against oxacillin or vancomycin, or Enterococcus against vancomycin. Read the results for the other antimicrobial disks then reincubate the plate for a total of 24 hours before reporting vancomycin or oxacillin.

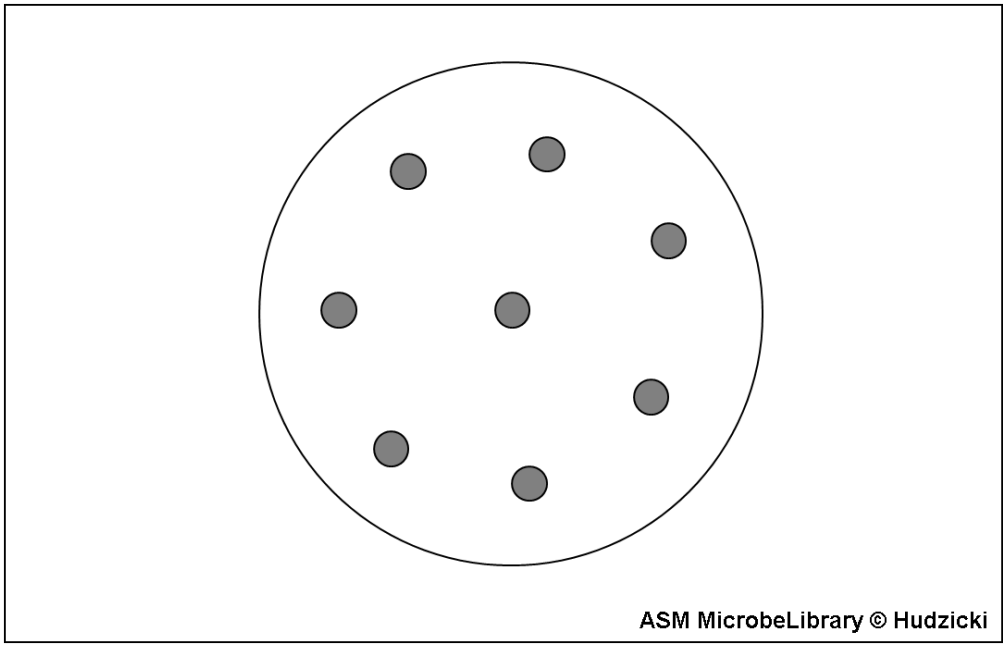


FIG. 7. Manual disk placement template for eight disks on a 100-mm plate. Place the MH agar plate on the figure above so that the edge of the plate lines up with the outer circle. Remove the lid from the plate and place one antibiotic disk on each dark gray circle. If fewer than eight antibiotics are used, adjustments can be made to the spacing of the disks. See "Additional Notes" disk placement for suggestions for printing this template.

Measuring zone sizes

1. Following incubation, measure the zone sizes to the nearest millimeter using a ruler or caliper; include the diameter of the disk in the measurement (Fig. 8, 9b).

2. When measuring zone diameters, always round up to the next millimeter.

3. All measurements are made with the unaided eye while viewing the back of the petri dish. Hold the plate a few inches above a black, nonreflecting surface illuminated with reflected light (Fig. 9a).

4. View the plate using a direct, vertical line of sight to avoid any parallax that may result in misreading.

5. Record the zone size on the recording sheet.

6. If the placement of the disk or the size of the zone does not allow you to read the diameter of the zone, measure from the center of the disk to a point on the circumference of the zone where a distinct edge is present (the radius) and multiply the measurement by 2 to determine the diameter (Fig. 10).

7. Growth up to the edge of the disk can be reported as a zone of 0 mm.

8. Organisms such as Proteus mirabilis, which swarm, must be measured differently than nonswarming organisms. Ignore the thin veil of swarming and measure the outer margin in an otherwise obvious zone of inhibition.

9. Distinct, discrete colonies within an obvious zone of inhibition should not be considered swarming. These colonies are either mutant organisms that are more resistant to the drug being tested, or the culture was not pure and they are a different organism. If it is determined by repeat testing that the phenomenon repeats itself, the organism must be considered resistant to that drug.

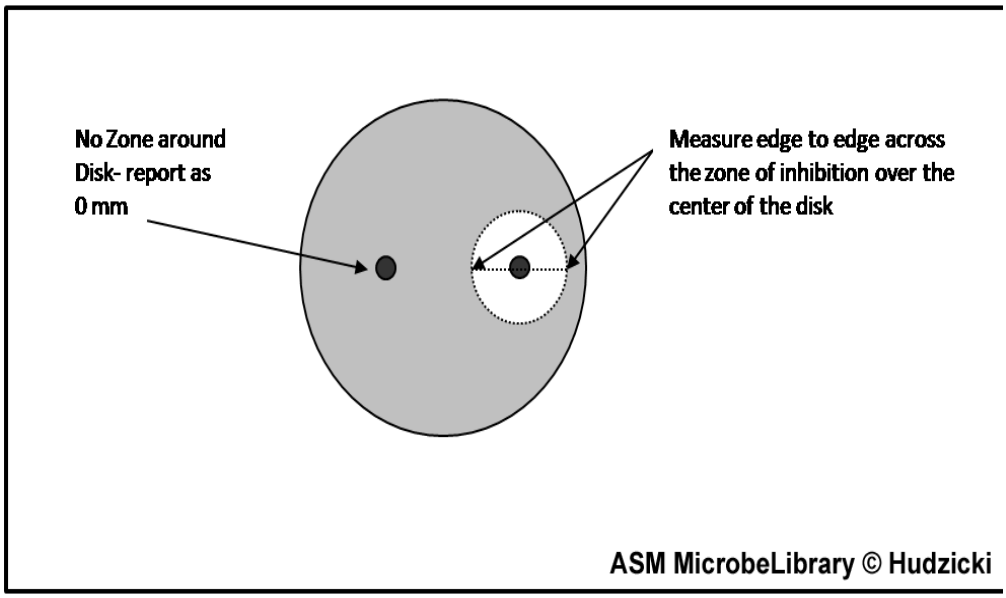
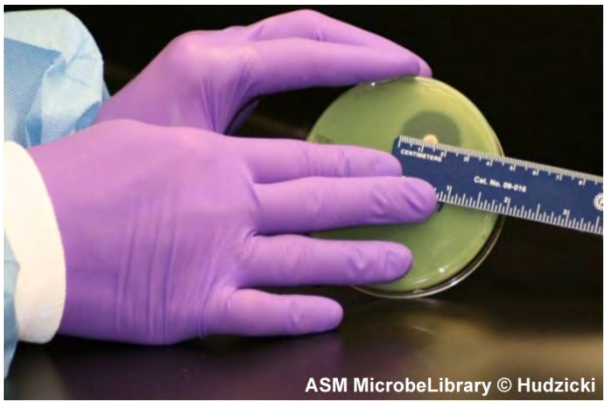
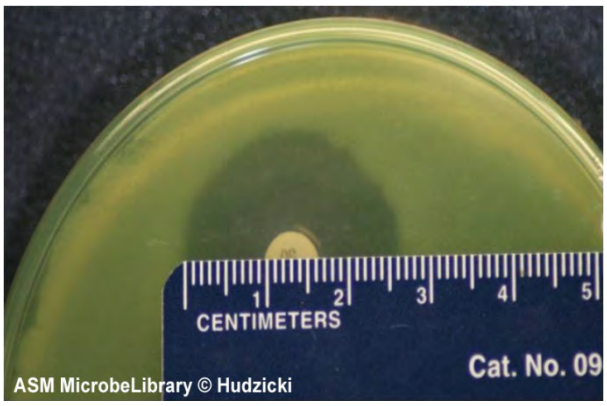


FIG. 8. Measuring zones of inhibition. Gray shading represents a confluent lawn of bacterial growth.
The white circle represents no growth of the test organism.



A



B

FIG. 9. Kirby-Bauer disk diffusion susceptibility test protocol, measuring zone sizes. (A) Using a ruler or caliper measure each zone with the unaided eye while viewing the back of the petri dish. Hold the plate a few inches above a black, nonreflecting surface illuminated with reflected light. (B) The size of the zone for this organism-antibiotic combination is 26 mm.

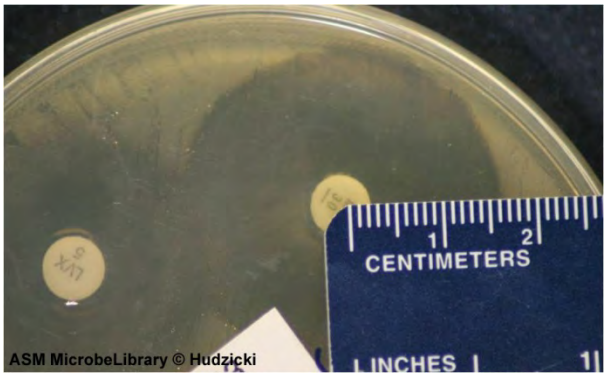


FIG. 10. Kirby-Bauer disk diffusion susceptibility test protocol, measuring zone sizes; an alternate method for measuring zones. If the zones of adjacent antibiotic disks overlap, the zone diameter can be determined by measuring the radius of the zone. Measure from the center of the antibiotic disk to a point on the circumference of the zone where a distinct edge is present. Multiply this measurement by 2 to determine the diameter of the zone of inhibition. In this example, the radius of the zone is 16 mm. Multiply this measurement by 2 to determine the zone size of 32 mm for this organism-antibiotic combination.

Measuring zone sizes

If the plate was properly inoculated and all other conditions were correct, the zones of inhibition should be uniformly circular and there will be a confluent lawn of growth.

If individual colonies are apparent across the plate, the inoculum was too light and the test must be repeated.

The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Do not use a magnification device to observe zone edges.

When measuring the zone of inhibition for organisms that swarm (e.g., Proteus sp.), ignore the thin veil of swarming growth in an otherwise obvious zone of inhibition.

With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter (3).

Interpretation and Reporting of the Results

1. Using the published CLSI guidelines, determine the susceptibility or resistance of the organism to each drug tested (Tables 1, 2, 3). Note that there are different charts for different organisms. Abbreviated charts specific for the author's suggested organisms and antimicrobial disks to use are provided below.

2. For each drug, indicate on the recording sheet whether the zone size is susceptible (S), intermediate (I), or resistant (R) based on the interpretation chart.

3. The results of the Kirby-Bauer disk diffusion susceptibility test are reported only as susceptible, intermediate, or resistant. Zone sizes are not reported to physicians.

Recommended Antimicrobial Disks and Interpretative Zone Sizes (3)_

This is a suggested battery of disks to use in the educational setting as it minimizes the different antimicrobials that would need to be purchased to test all three groups of organisms. Educators may select other drugs from the CLSI charts, as appropriate for their situation.

TABLE 1. Zone diameter interpretative standards for Staphylococcus species (3)

***Staphylococcus* species**
(Zone Diameter, nearest whole mm)

	Resistant	Intermediate	Susceptible
Cefazolin (30 µg)	≤14	15-17	≥18
Clindamycin (2 µg)	≤14	15-20	≥21
Erythromycin (15 µg)	≤13	14-22	≥23
Gentamicin (10 µg)	≤12	13-14	≥15
Oxacillin (1 µg)	≤10	11-12	≥13
Penicillin G (10 µg)	≤28	--	≥29
Tobramycin (10 µg)	≤12	13-14	≥15
Vancomycin (30 µg)	--	--	≥15

TABLE 2. Zone diameter interpretative standards for Pseudomonas aeruginosa and other nonfermenting gram-negative rods (3)

***Pseudomonas aeruginosa* and other non-fermenting Gram Negative Rods**
(Zone Diameter, nearest whole mm)

	Resistant	Intermediate	Susceptible
Amikacin (30 µg)	≤14	15-16	≥17
Cefoperazone (75 µg)	≤15	16-20	≥21
Cefotaxime (30 µg)	≤14	15-22	≥23
Gentamicin (10 µg)	≤12	13-14	≥15
Piperacillin (100 µg)	≤17	--	≥18
Tetracycline (30 µg)	≤14	15-18	≥19
Ticarcillin (75 µg)	≤14	--	≥15
Tobramycin (10 µg)	≤12	13-14	≥15

TABLE 3. Zone diameter interpretative standards for E. coli and other enteric gram-negative rods (3)

***E. coli* and other enteric Gram Negative Rods**
(Zone Diameter, nearest whole mm)

	Resistant	Intermediate	Susceptible
Amikacin (30 µg)	≤14	15-16	≥17
Ampicillin(10 µg)	≤13	14-16	≥17
Cefazolin (30 µg)	≤14	15-17	≥18
Gentamicin (10 µg)	≤12	13-14	≥15
Tetracycline (30 µg)	≤14	15-18	≥19
Ticarcillin (75 µg)	≤14	15-19	≥20
Trimethoprim (5 µg)	≤10	11-15	≥16
Tobramycin (10 µg)	≤12	13-14	≥15

SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more

information, read the laboratory safety section of the ASM Curriculum Recommendations: Introductory Course in Microbiology and the Guidelines for Biosafety in Teaching Laboratories.

REFERENCES

1. **Bauer, A. W., D. M. Perry, and W. M. M. Kirby.** 1959. Single disc antibiotic sensitivity testing of Staphylococci. A.M.A. Arch. Intern. Med. 104:208–216.
2. **Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 36:493-496.
3. **Clinical Laboratory Standards Institute.** 2006. Performance standards for antimicrobial disk susceptibility tests; Approved standard—9th ed. CLSI document M2-A9. 26:1. Clinical Laboratory Standards Institute, Wayne, PA.
4. **Difco.** 1984. Difco manual, 10th ed. Difco Laboratories, Detroit, MI.
5. **Kirby, W. M. M., G. M. Yoshihara, K. S. Sundsted, and J. H. Warren.** 1957. Clinical usefulness of a single disc method for antibiotic sensitivity testing. Antibiotics Annu. 1956-1957:892.
6. **Winn, Jr., W., et al.** 2006. Konemann's color atlas and diagnostic text of microbiology, 6th ed., p. 945–1021. Lippencott Williams & Wilkins Publishers, Philadelphia, PA.
7. **Jorgensen, J. H., and J. D. Turnidge. 2007.** Susceptibility test methods: dilution and disk diffusion methods, p. 1152–1172. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Pfaller (ed.), Manual of clinical microbiology, 9th ed. ASM Press, Washington, D.C.

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