

HiPer[®] Antibiotic Sensitivity Teaching Kit

Product Code: HTM002

Number of experiments that can be performed: 15

Duration of Experiment:

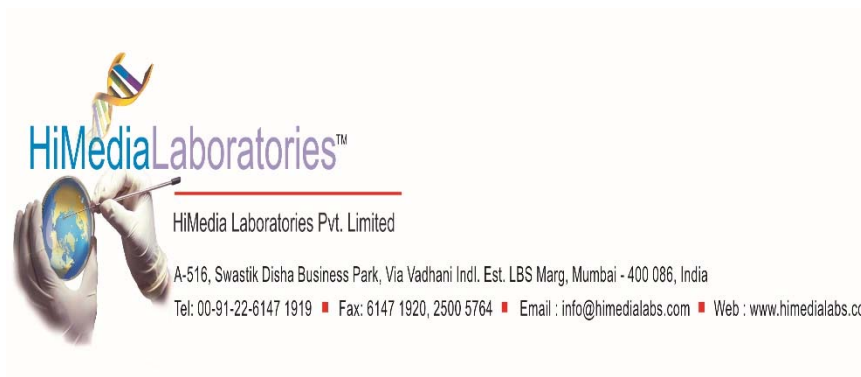
Day 1: Preparation of media and revival of strains

Day 2: Protocol

Day 3: Observations and Results

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
 - Store all the culture stabs and antibiotic discs at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)



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Aim:

To determine the sensitivity of bacteria against various antibiotics.

Introduction:

Antibiotic sensitivity describes the susceptibility of bacteria to various antibiotics. Clinical microbiologists have a major role to play in prescribing antibiotics for either treatment or prophylaxis of infection. The antibiotics for therapy are selected after performing Antibiotic Susceptibility Test (AST). It is often done by the Kirby-Bauer method in which antibiotic impregnated discs are used to test the susceptibility of any bacterial strain to a specific antibiotic.

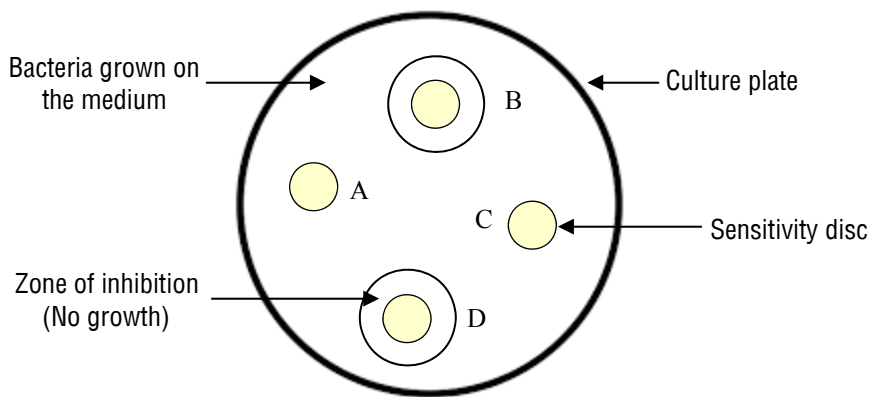


Fig 1: Sensitivity of bacteria to antibiotics B and D but not to A and C

Principle:

HiPer[®] Antibiotic Sensitivity Teaching Kit allows classification of bacterial strains as susceptible, resistant, or intermediate to various antimicrobial agents. In this method, the antibiotic impregnated discs are placed on the Mueller Hinton Agar plates on which the bacterial culture is spread. As the antibiotic impregnated disc comes in contact with the moist agar surface, water is absorbed in the disc paper and the antibiotic diffuses out in the surrounding medium. As the distance from the disc increases, there is a logarithmic reduction in the antibiotic concentration which creates a gradient of drug concentration in the agar medium surrounding each disc. Though the diffusion of drug occurs, the bacteria that are inoculated on the agar surface are not inhibited by the concentration of antimicrobial agents but continue to multiply until a lawn of growth is visible. No growth occurs in the areas where the concentration of drug is inhibitory thus forming a zone of inhibition. Thus when an organism is sensitive to any antibiotic, a clear zone appears around that specific disc where the growth has been inhibited (zone of inhibition) whereas if an organism is resistant no clear zone of inhibition appears.

Sensitive (S): An organism is called 'sensitive' to a drug when the infection caused by it is likely to respond to the treatment with that specific drug at the recommended dosage.

Intermediately sensitive (I): It is applicable to organisms that are moderately sensitive to an antibiotic that can be used for treatment at a higher dosage and as a result leads to uncertain therapeutic effect.

Resistant (R): An organism is called 'resistant' to a drug when the organism does not respond to a given drug irrespective of the dosage.

The diameter of the zone of inhibition surrounding the antibiotic disc is measured to determine whether the microorganism is sensitive or resistant to a particular antibiotic. The zone size depends on:

1. The rate of diffusion of the antibiotic through agar
2. The concentration of the antibiotic present in the disc
3. The degree of sensitivity of the microorganism
4. The growth rate of the bacterium

Thus, by performing AST clinicians can select the most appropriate antibiotic for treatment. Also various microbial strains can be studied for their susceptibility to various antibiotics.

Kit Contents:

This kit can be used to determine the sensitivity of bacteria against various antibiotics.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			15 PR	
1		Cultures		
a	TKC029	<i>Escherichia coli</i>	1	2-8°C
b	TKC030	<i>Staphylococcus aureus</i>	1	2-8°C
c	TKC031	<i>Pseudomonas aeruginosa</i>	1	2-8°C
2		Antibiotic discs		
a	SD006	Chloramphenicol	50*	2-8°C
b	SD037	Tetracycline	50*	2-8°C
c	SD017	Kanamycin	50*	2-8°C
d	SD016	Gentamycin	50*	2-8°C
e	SD045	Vancomycin	50*	2-8°C
3	PW005	Sterile cotton swabs	50 Nos.	RT
4	M173	Mueller Hinton Agar	65 g	RT
5	TKC064	Sterile Saline	50 ml	RT
6	TKC129	Applicator	1 No.	RT

* Antibiotic discs are provided in a cartridge. One cartridge contains 50 antibiotic discs.

Materials Required But Not Provided:

Glass wares: Sterile test tubes, Conical flasks, Sterile Petri plates

Other requirements: Incubator, Sterile forceps, Micropipettes, Tips, Inoculation loop, Measuring cylinder, Distilled water.

Storage:

HiPer® Antibiotic Sensitivity Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store cultures and antibiotic discs at 2-8°C. Other kit contents can be stored at room temperature (15-25°C).

Important Instructions:

1. Read the entire procedure carefully before starting the experiment.
2. Perform all the microbiological experiments under aseptic conditions.
3. Thaw all refrigerated samples before use.
4. **Preparation of Mueller Hinton (MH) Agar plates (150 ml):** Dissolve 5.7 g of MH agar into 150 ml of sterile distilled water. Sterilize by autoclaving and allow the media to cool down to 40-45 °C and pour on sterile petri plates.

Procedure:

Day 1: Revival of Strains

1. Open the vials containing cultures (*E.coli*, *S.aureus*, *P.aeruginosa*) and resuspend the pellet individually with 0.25 ml of LB broth.
2. Pick up a loopful of culture and streak onto MH agar plate.
3. Incubate overnight at 37°C.

Day 2: Antibiotic Sensitivity Test

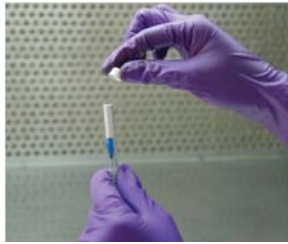
1. Pick up a single colony of each strain (*E. coli*, *S. aureus* and *P. aeruginosa*) from the MH agar plate and inoculate into three sterile test tubes respectively containing 1 ml of sterile saline. Mix the tubes thoroughly.
2. Take a sterile cotton swab and dip it into the *E. coli* labeled test tube.
3. Spread the cotton swab containing the *E. coli* culture evenly onto the *E. coli* labeled Mueller Hinton Agar plate.
4. Similarly, follow steps 2 and 3 for *S. aureus* and *P. aeruginosa*.
5. Allow the plates to dry for 5 minutes.
6. Using applicator place the antibiotic discs onto the surface of the *E. coli* Mueller Hinton Agar plate as shown in figure 2.

Note: Press the antibiotic discs gently with forceps onto the surface of the *E. coli* Mueller Hinton Agar plate to ensure firm contact of the antibiotic disc with the agar surface.

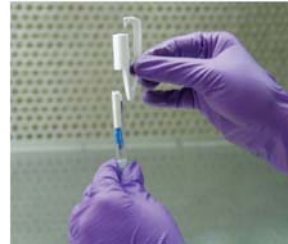
7. Similarly follow steps 9 and 10 for *S. aureus* and *P. aeruginosa*.
8. Incubate all the plates at 37°C for 24 hours



1) Cartridge containing antibiotic discs



2) Open the cap of the cartridge



3) Put the applicator onto the cartridge



4) Fix the applicator tightly



5) Press the knob of the applicator as shown



6) Place the antibiotic disc onto the plate as shown above

Fig 2: Placement of the antibiotic discs from the cartridge onto the plate

Standard Zone Size Interpretative Chart:

Sr. No.	Antimicrobial Agent	Concentration (in mcg)	Resistant (mm or less)	Intermediate (mm)	Sensitive (mm or more)
1	Chloramphenicol	30	12	13-17	18
2	Gentamicin	30	12	13-14	15
3	Kanamycin	30	13	14-17	18
4	Tetracycline	30	14	15-18	19
5	Vancomycin	30	-	-	15

Observation and Result

Observe the plates for zone of inhibition to the antibiotics surrounding the respective discs after 24 hours.



Measure the zone of inhibition by placing a ruler under the petri plate. Place the metric ruler across the zone of inhibition, at the widest diameter, and measure from one edge of the zone to the other edge and tabulate the results as follows:

Table 2: Measurement of zone of inhibition

Sr. No.	Antibiotics	Zone of inhibition (in mm)		
		<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
1	Chloramphenicol			
2	Gentamycin			
3	Kanamycin			
4	Tetracycline			
5	Vancomycin			

Compare the results obtained with that of the standard to determine the susceptibility of each organism to each of the five antibiotics and record them as follows:

S- Sensitive

I-Intermediate

R- Resistant

Table 3: Susceptibility of each organism

Sr. No.	Antibiotics	Organism		
		<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
1	Chloramphenicol			
2	Gentamycin			
3	Kanamycin			
4	Tetracycline			
5	Vancomycin			

Interpretation:

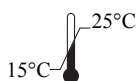
The appearance of a zone of inhibition surrounding the disc is indicative of susceptibility of the organism to the respective antibiotic. By comparing the diameter of the zones to the standard zone size interpretative chart, one may determine if the test organism is sensitive, intermediately sensitive or resistant to the antibiotic.

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1	No clear zone of inhibition	Antibiotic may have been degraded	The antibiotic discs should be stored at 2-8°C
		No even bacterial growth observed	Ensure that the organism is evenly streaked on the plate

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com



Storage temperature



Do not use if package is damaged



HiMedia Laboratories Pvt. Limited,
23 Vadhani Industrial Estate,
LBS Marg, Mumbai-86, MS, India

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