International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 24-08-2021, Accepted: 08-09-2021, Published: 24-09-2021
Volume 6, Issue 5, 2021, Page No. 836-839

# Agar well diffusion: A prominent method for *In vitro* screening of antimicrobials

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**Abstract**
Antimicrobial fend off millions of casualties every year. Unsuitable prescription and overdoing of antimicrobial have steered resistance as a global health emergency and results in 700,000 mortalities a year. The World Health Organization cogitates antimicrobial resistance as a universal threat to human health. Novel resistance mechanisms are evolving and dispersing globally, bullying our capability to treat infectious diseases. The increasing difficulty in treating bacterial and fungal infections is due to the perpetual issue of microbial resistance to antibiotics. The primary goal of antimicrobial research insists on avoiding resistance by overcoming existing bacterial cell processes and reducing side effects. Agar well diffusion method involves a simple and efficient experimental procedure. To infiltrate the agar plate, it is smeared with a dissemination of the microbial inoculum across the whole surface. Following this, a well is aseptically poked with a sterile cork borer and the test/standard solution is put into the well. Next, agar plates are cultured in context to the kind of microorganism being tested. The antimicrobial ingredient permeates the agar medium and interferes with the development of the tested microbial strain.

**Keywords:** agar, well, antimicrobial, testing, diffusion

## Introduction
Antimicrobial refers to a class of antibiotics that are used against microorganisms and may either kill or suppress them. There are a growing number of antibiotic medicines that bacterial and fungal diseases are quickly becoming resistant [1,2]. Unjustified antibiotic use as well as genetic factors specific to bacteria have resulted in antimicrobial resistance. Antimicrobial resistance is a major public health issue because it reduces the efficacy of antimicrobial therapies, resulting in higher morbidity, mortality and health care costs [3, 4]. Antimicrobial resistant [5, 6] organisms and their effects seem to be uncontrolled. The primary goal of antimicrobial research insists on avoiding resistance by overcoming existing bacterial cell processes and reducing side effects. A ray of hope is the safe use of antibiotics and the production of new antimicrobials. Thus increasing difficulty in treating bacterial and fungal infections is due to the perpetual issue of microbial resistance to antibiotics, which requires the development of new generations of antimicrobial medicines [7,8].

## Microbiological Screening
A microorganism test determines the potency of antibiotics in medications by utilizing microbes. The test relies on the relationship between use of an antibiotic on microorganisms and its growth. The zone of microbial growth inhibition measurement is the basis of the technique. The standard antibiotic that's already known to have an effect on microorganisms is used as a comparison. A standard preparation is a genuine sample of an antibiotic whose strength has been accurately established using an international standard. The potency may be measured in terms of an international units or in microgram per mg of pure antibiotic. In the face of limited antibiotic discovery from microorganisms, the improvement of screening techniques used to find natural antimicrobials is crucial. Screening against two bacterial species, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, using agar diffusion methods (well and disc) demonstrated that the disc variant is much less sensitive than the well procedure [9].

## Agar well diffusion method
Indian Pharmacopoeia mentions components that are used to make the necessary media for the production of test organisms. Phosphate buffer solutions are prepared by dissolving dipotassium hydrogen phosphate and potassium dihydrogen phosphate in water and the resulting solution is used as a buffer. For the Standard Preparation of a particular antibiotic, precisely weighed and dried amount is dissolved in anappropriate solvent (or as described in the Indian Pharmacopoeia) and then diluted to the desired concentration. Preparation of the solution of the item being tested ("unknown"), potency is estimated per unit weight or per unit volume and a stock solution is prepared using the same diluent as the standard preparation. Only one level of the unknown is needed for the assay with 5 levels of the standard because the concentration of the unknown is expected to be equal to the median of the standard.

## Investigational particulars
In the middle of a sterile petri plate, one milliliter of freshly grown bacterial or fungal culture is pipetted. A Muller Hi PDA (molten cooled) inoculum is then put onto the Petri plate and stirred well. After solidifying, inoculated agar plates are used to drill well cavities using a sterile cork borer (6 mm in diameter). A 100 µL of 20 % (w/v) may be applied to the corresponding wells for each of the test. The test is allowed to be thoroughly absorbed into the agar by placing the plates in the refrigerator for 30 minutes. Following incubation at 37°C for 18 hours, the presence of antibacterial properties is verified by determining the size of the zones of inhibition (including the width of the wells). A 10% DMSO solution may be used as a negative control [10].

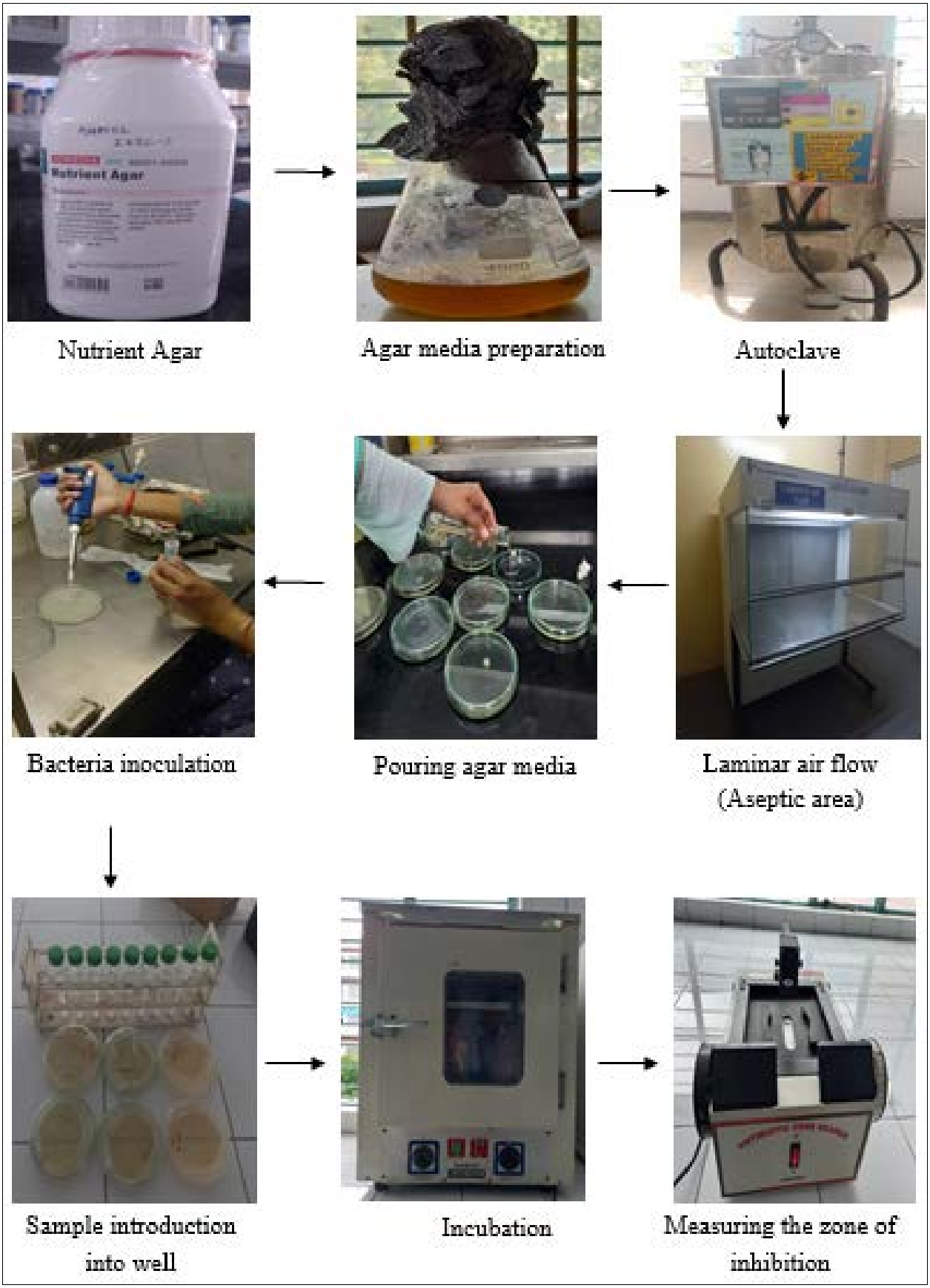


**Fig 1:** A typical approach for agar well diffusion method

**Minimum inhibitory concentration determination**

Dissemination in agar may be used as a standard method for minimum inhibitory concentration detection in solid medium. A bacterial culture is inoculated into agar plates and the dose of antibiotic solutions in wells is varied to treat the bacterial. Wells inject antibiotics into the agarose, creating zones where bacterial growth is inhibited. These patches become bigger as the antibiotic strength goes higher. The minimum inhibitory concentration (MIC) value is calculated as the zero intercept of a straight interpolation

of the squared size of zone of inhibition (x) and concentration (c) [11].

$$\ln (MIC) = \ln(c) - \frac{x^2}{4Dt}$$

D = diffusion coefficient
t = amount of time for the antibiotic diffusion.

**Experimental landfills**
Petri dishes are filled to a depth of 3-4 mm and the inoculated culture medium to the dishes or plates is applied at a temperature between 40 °C and 50 °C. The two layer nutritional agar may be inoculated using the top layer alone. When appropriate, plates are left out for 1 to 4 hours at room temperature or 4 °C before putting them in the incubator to ensure equal pre incubation diffusion time. Placing standard antibiotic/test in the agar plates within 15 minutes of generating the bacterial lawn is crucial when doing diffusion tests. In order to incubate the plates, it is recommended to place them into the incubator inside 15 minutes after applying the standard antibiotic/test [12]. After incubation for abot 18 hours, accurate measurements of the circular inhibitory zones diameters is plotted against the drug concentration. To get the standard curve, a straight line between these locations is drawn. Through concentration of the unknown, the diameter size may be acquired from this graph.

**Finest consequence settings**
A nonhomogeneous distribution of test/standard in water is possible by utilizing the agar overlay approach, where a reservoir is a well. For better results, the inoculation system may be maintained at a low temperature prior to incubation, which helps the movement of the culture media and expands the inhibition zone. The techniques is inappropriate when the specimen is not very soluble in water like nonpolar compounds. This technique is effective for determining the sensitivity of the test organism and some studies claim a correlation between MIC values and inhibition diameters [13]. Theory of regression lines links inhibitory halos and MIC (thresholds of infectiousness) [14]. The technique is not suitable when testing materials are essential oils or complex mixtures that have a lesser antibacterial activity and greater diffusion power.

**Pros and cons**
The findings of the diffusion test is generally qualitative as it categorise whether a sample is sensitive, moderate or refractory [15]. Smaller sample size is utilised in the screening process. It provides the opportunity to test five or six substances against a single microbe. However, it is not best for lipophilic samples. Pure compounds are well suited for preliminary screening using diffusion techniques. It is never acceptable to use harmful germs that are not well connected to the rest of the population. To calculate the degree of success, + (increase) or - (reduction) values may be determined, which can then be compared to the dilution techniques [16].

**Conclusion**
The antimicrobials screening methods has become a need due to the rise in bacterial resistance, the return of previously managed illnesses and the introduction of emerging infections. Agar well diffusion method involves a simple and efficient experimental procedure.

**Conflict of interest**
The authors declare no conflict of interest.

**Acknowledgments**
Juhee Ansari is a M. Pharm (Pharmaceutical Chemistry) student at Department of Pharmacy. Authors are grateful to the Integral University, Lucknow (Manuscript communication number: IU/R&D/2021-MCN0001228) for providing the necessary facilities for carrying out this work.

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