Minimum Inhibitory Concentration

The MIC is the lowest concentration of an antimicrobial agent that visually inhibits the growth of a microorganism under defined experimental conditions. MIC values lower than the breakpoint are interpreted as susceptible results and those higher as resistant for treatment guidance. MIC breakpoint values are vital in categorizing susceptibility group for *In vitro* antimicrobial susceptibility testing and clinical interpretation. Understanding the concept of MIC and its relations to the interpretive breakpoint is one of the major hassles to microbiologists and clinicians. Since the pharmacokinetics of antimicrobial agents can be different two agents with the same MIC value for a pathogen may have totally different interpretations because they have different breakpoints. MIC testing is a very valuable quantitative assay tool for evaluating the pathogenic microorganism's degree of susceptibility and to detect the specific resistance mechanism. Today clinical microbiology laboratories can provide MIC testing services and in many cases exact values for determining the therapy for the individual patient. Selection of the most effective antimicrobial agent and dosing regimen for serious infection will help in eliminating the pathogens and minimize resistance selection and decrease mortality.

HiMedia has adopted this system in the form of HiComb™ (MD), which is based on innovative disc diffusion and gradient-based technique, essentially with a wide choice of antibiotics. This system is developed using dry chemistry technology and consists of two comb-shaped strips made of an inert material with antibiotic discs at the ends and the Ezy MIC™ Strip is based on gradient technology and the combination of dilution & diffusion principles for Testing of Susceptibility. This method generates the MIC values of a given antibiotic in µg/ml, which will inhibit the growth of a particular microorganism under the specified set of experimental conditions. The MIC value obtained from this method can be compared to the standardized CLSI procedure.

Parameters Affecting the Performance of Susceptibility Discs

1) The first and most important parameter affecting the performance of the discs is TEMPERATURE. This includes the transportation, storage & handling of discs. It should be made sure that the discs should be transported in cold chain. The user should be instructed not accept the discs if they do not receive them in ice pack. The customers on the receipt of the discs should also immediately place them at 2-8°C. The discs which are not to be used in near future should be stored at -20°C. Antimicrobial discs must be handled very carefully as many of the antimicrobial agents are temperature sensitive. Unopened/ used cartridges (containing discs) should be allowed to equilibrate to room temperature before opening. Discs must be stored with desiccant in containers with tightly fitting covers and refrigerated after each use. Even when the discs are removed, it should be made sure that they are not left out for too long and that if a burner is used for testing it should not be placed around the vicinity of the burner.

2) Second is THE MEDIUM USED IN TESTING AND ITS PREPARATION. The activity of the antimicrobial agent, growth rate of the test organism and the rate of diffusion of the antimicrobial agent are largely influenced by the composition of the test medium. The agar must allow free diffusion of the antimicrobial from the disc. Hence it is advisable to pour the plates to a depth of 3 to 5 mm. Smaller zone(s) of inhibition may appear due to the increased agar depths and thus false negative results may be interpreted. Variation in ionic strength and pH affects the zone sizes. The effect of antimicrobials like Aminoglycosides, Polymyxins and Tetracyclines is affected by free electrolyte content of the medium. For e.g. **Mueller - Hinton Agar should be monitored to make sure that it contains the acceptable concentrations of divalent cations such as Ca2+ and Mg2+, as the increased concentration of these results in decreased activity of Aminoglycosides and Tetracyclines (decreased concentrations of Ca2+ and Mg2+ have the opposite effect). Higher concentrations of thymine or thymidine result in decreased activity of Sulphonamides and Trimethoprim etc. Low or acidic pH results in the decreased activity of Aminoglycosides, Erythromycin and Clindamycin i.e. false resistance. However, the low pH results in increased activity of Tetracyclines. Glucose (dextrose) will enlarge the zones of antibiotics against the organisms; which are unfavorably affected by a drop in pH due to sugar fermentation. Blood, if added to the medium can reduce the zone size of protein-bound antimicrobials like Fusidic Acid. To avoid the antagonism of Trimethoprim and Sulphonamides, the levels of thymine and thymidine should be controlled and monitored**. HiMedia's range covers a number of culture media recommended for determining antimicrobial susceptibility using agar disc diffusion technique. The culture media mainly used for these tests are Mueller Hinton Agar (M173), Mueller Hinton HiVeg™ Agar (MV173), Mueller Hinton Agar No.2 (M1084), Mueller Hinton HiVeg™ Agar No.2 (MV1084). HiSensitivity Test Agar (M485), HiSensitivity Test HiVeg ™ Agar (MV485), HiSitest Agar (M485A). Wilkins Chalgren Anaerobic Agar (M832). Diagnostic Sensitivity Test (D.S.T.) Agar (M502) and G.C. Agar (M434) with added FD022 & FD025. The medium plates that are prepared should be as per manufacturer's instruction. The plates poured should have an even surface and depth.

Mueller Hinton Agar (M173)

plate showing zones of inhibition when impregnated antimicrobial sensitivity discs are used

3) Thirdly THE INOCULUM OF THE CULTURE used in the test, be it a standard ATCC strain or a clinical isolate. This is one of the most critical factors influencing the antimicrobial susceptibility test. It is advisable to use a technique, which always yields a uniform suspension of the correct number of organisms i.e. 10^5 - 10^6 cells/ ml, as a more dense culture will give zones smaller then expected and vice-versa. Even if the density of culture is adjusted, due to improper inoculation false results could be obtained. Inoculation method includes the correct and even swabbing of plates & correct placement of discs.