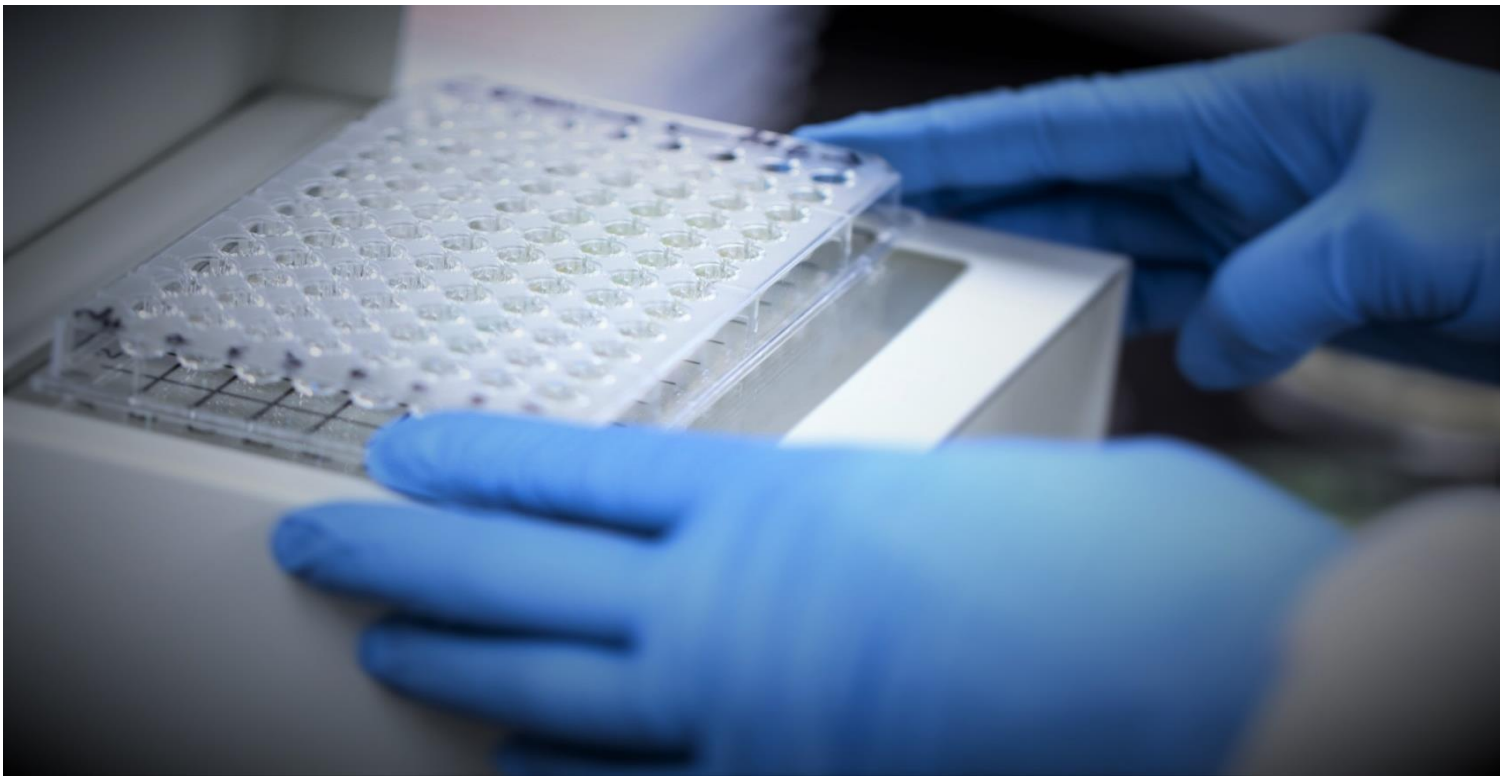

Minimum Inhibitory Concentration (MIC)

For many years, the Kirby-Bauer disk diffusion method accounted for most susceptibility tests. Using the Kirby-Bauer method, a zone of inhibition (where bacteria did not grow) could be identified and the level of antibiotic resistance displayed by the bacteria could be determined[1]. However, because the Kirby-Bauer method of testing susceptibility is incapable of producing quantitative results, it has been replaced by the MIC method. MIC assays generate a value in µg/mL for the exact, lowest concentration of the antimicrobial agent that prevents the visible growth of bacteria.

At EPS, our microbiologists routinely perform MIC assays to evaluate antibiotic efficacy. We frequently test various antimicrobial compounds including plant derivatives, proteins, coatings, etc. with known, resistant, bacterial strains including "ESKAPE" pathogens and MRSA strains in order to determine antimicrobial susceptibility. This guide briefly summarizes MICs, how to report results, and how MICs are used.

- About MICs
- Reporting MICs
- How MICs are Used

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About MICs

The minimum inhibitory concentration is the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism after overnight incubation in broth[2]. This precise method of susceptibility testing allows for quantification of the exact concentration (in µg/mL) of the antibiotic (or client selected test article) needed to inhibit the bacterial growth. Comparing an MIC value to the CLSI breakpoint values allows to determine whether a bacterium is susceptible, intermediate, or resistant to an antibiotic at certain concentrations. This information is most often used as a research tool to determine the in vitro activity of new antimicrobials and screen for the best candidates.

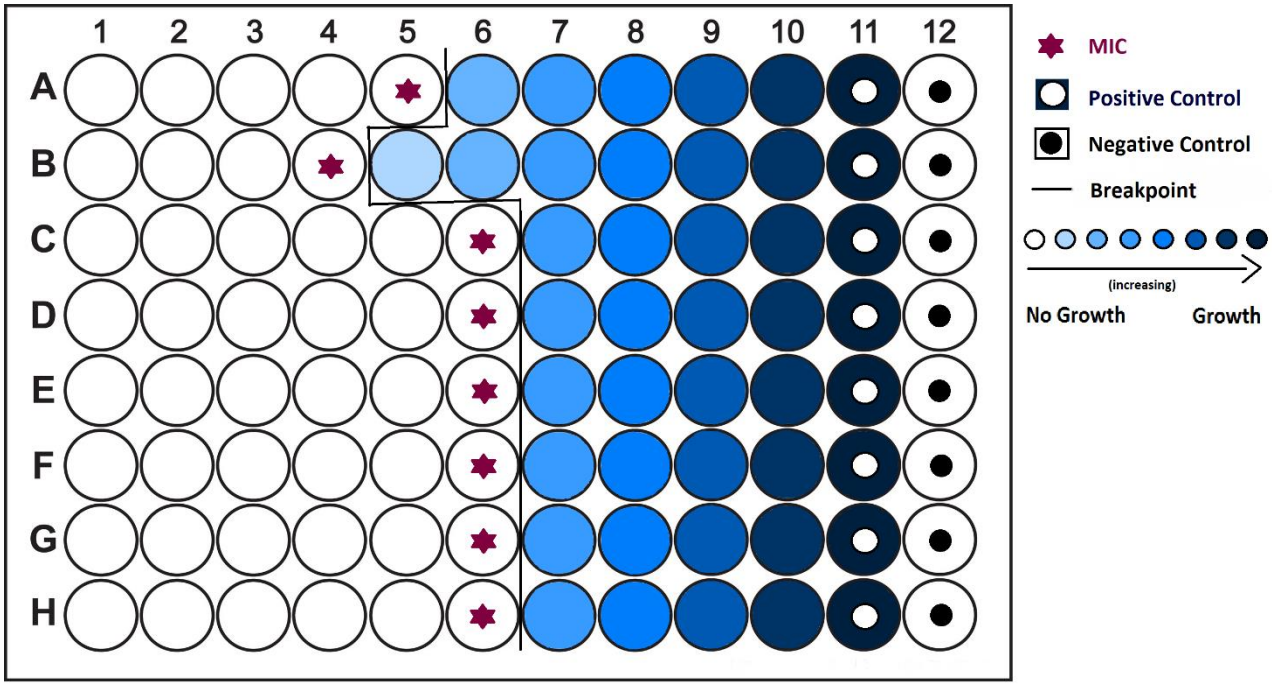

Figure 1. Example MIC microtiter plate.

The image (above) depicts a typical microtiter plate for an MIC assay. The wells in the columns 1-10 at the top of the plate contain antimicrobial agent of varying concentrations in decreasing order (1 is the highest concentration and 10 is the lowest concentration). The wells in columns 11 and 12 contain positive (no antimicrobial agent) and negative controls (no bacteria), respectively. The positive and negative controls are used to validate the accuracy of the tested antimicrobials. The wells in rows A through H on contain various antimicrobial agents being tested.

The white circles represent no bacterial growth, meaning that the antimicrobial succeeded in inhibiting the bacteria growth for a certain concentration. The blue circles depict bacterial growth—the lighter shades indicate less growth and the darker shades indicate more growth. The vertical black line for each row represents the breakpoint--concentration at which the bacteria is no longer susceptible to the antimicrobial but becomes resistant. The purple stars indicate the MICs for each antimicrobial—the lowest concentration at which the bacteria are susceptible to selected antimicrobial.

Reporting MICs

MICs are reported as the lowest concentration (µg/mL) of an antibiotic that inhibits visible bacterial growth. Comparing an MIC value to the breakpoint value reveals whether bacteria are susceptible, intermediate, or resistant to a specific antibiotic. The result "susceptible" indicates that the antibiotic effectively inhibited in vitro bacterial growth at the MIC, which would in turn indicate a potential therapeutic success in clinical trials. "Intermediate" signifies that the antibiotic inhibited in vitro bacterial growth at a high dosage of the drug but implies that there are unknown therapeutic effects. "Resistant" bacteria are not inhibited by achievable concentrations of the antibiotic and/or fall in the range where specific microbial resistance mechanisms are likely. As a result, there is a high likelihood of therapeutic failure and other candidates should be considered.

			MIC (µg/mL)											
Organism Description	**Isolate #**	**Phenotype**	**Ciprofloxacin**		**Clindamycin**		**Erythromycin**		**Oxacillin**		**Trimeth/ Sulfa**		**Vancomycin**	
Staphylococcus aureus	1674625	MRSA	0.5	S	0.12	S	>8	R	>8	R	≤0.25	S	1	S
Staphylococcus aureus	1674631	MRSA	>16	R	>4	R	>8	R	>8	R	≤0.25	S	1	S
Staphylococcus aureus	1674612	VISA							4	R			8	I

Figure 2. Example MIC Report.

The table above is an MIC report of various strains of *Staphylococcus aureus* against antibiotics such as ciprofloxacin, clindamycin, erythromycin, oxacillin, sulfamethoxazole/trimethoprim, and vancomycin. The MICs of each strain against each antibiotic are represented, along with S (susceptible), I (intermediate), or R (resi stant) indications next to the number. The MIC is determined through the assay, but the interpretation (S, I, or R) is determined by comparing the MIC to the CLSI breakpoint values that indicate the extent of bacterial activity. The breakpoint for a specific antibiotic (with a specific bacterial strain) is the value of the concentration that determines susceptibility and resistance. (Breakpoints differ by drug and bacterial strain.) If the MIC is greater than the breakpoint value, then the bacteria is resistant to the antibiotic. If the MIC is less than or equal to this value, then, the bacteria is considered susceptible to the antibiotic.

How MICs are Used

Typically, MICs are used as screening assays and are performed on a large number of client selected test articles. Once successful candidates are identified through the MIC, minimum bactericidal concentrations (MBCs) assays are performed. MBC is defined as the lowest concentration of antimicrobial that results in 99.9% reduction in the initial microbial density[3].The MBC is a complimentary assay to an MIC. In an MIC assay the lowest level of antimicrobial agent that inhibits bacterial growth is determined, while MBC determines the lowest level of antimicrobial agent that results in microbial death[4]. This means that even if a particular MIC assay shows inhibition, culturing the bacteria in media might still result in organism proliferation because the antimicrobial did not completely kill the bacteria. Thus, an MBC assay is necessary to determine if an antimicrobial was truly bactericidal and killed 99.9% of the bacteria.

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