

UAB MOPA
Optochin Problem
September 2012

Recently, we have noted variations in the activity of optochin, but the source of this variation is unclear. Thus, we have instituted a policy to titrate each new batch of optochin to ensure adequate performance. For each strain of interest (optochin resistant **and** optochin sensitive strains should be tested), optochin should be tested at 0.5X, 1X, and 2X of the desired assay concentrations, following the protocol described for bacteria stock characterization in section 1.C.4 of *Protocol for multiplexed opsonophagocytic killing assay (UAB-MOPA) for antibodies against Streptococcus pneumoniae*. Optochin sensitive strains should not grow at 0.5X and optochin resistant strains should grow normally at 2X.

If these conditions are not met, the optochin concentration should be adjusted accordingly. For example, we had a situation where EMC23F (an optochin sensitive strain) grew at 2 mg/L of optochin (the optochin concentration used previously). Titration data indicated that EMC23F (as well as SPEC1, STREP5, TREP6A, SPEC6B, EMC9V, STREP14, TREP19A, and SPEC19F) would not grow at 4 mg/L; and the optochin resistant strains OREP3, OREP4, OREP7F, and OREP18C would grow at 16 mg/L. In this situation, the assay concentration of OP was adjusted to 8 mg/L.

If microcolonies are noted on optochin-containing plates (especially if EMC23F is included in that assay group), these titration experiments should be performed to ensure that the concentration of optochin is adequate.