

SIENA MCGOVERN*, JORDAN GIBSON*, EMILY YOUNG, & DEANNA M. SCHMITT, Department of Biological Sciences, West Liberty University, West Liberty, WV 26074. Stability of resazurin in solution and in bacterial growth media

Resazurin has been identified as a novel antibiotic against the human pathogens, *Neisseria gonorrhoeae* and *Francisella tularensis*. During routine antibiotic susceptibility assays, variations in vitro efficacy of resazurin have been noted, particularly in the minimal inhibitor concentration (MIC). The stability of this compound in aqueous solution is not known therefore, we hypothesize degradation of the antibiotic occurs during prolonged storage resulting in inconsistent MIC measurements. To test this hypothesis, solutions of resazurin at 22mM (5.5 mg/ml) were stored at 4°C for up to three months. At different time points, these solutions were then used in broth microdilution assays to determine the MIC of resazurin. The average MIC of resazurin was measured to be 0.69 µg/ml, only varying by a factor of two over the three-month period. Next, we sought to measure the stability of resazurin in chocolate agar, growth media commonly used for the cultivation of *N. gonorrhoeae* and *F. tularensis*. Here, we prepared chocolate agar plates supplement with increasing concentrations of resazurin and stored these plates for up to three months prior to performing standard agar dilution assays to determine the MIC. The MIC of resazurin in chocolate agar was 2-4 fold higher than the MIC calculated in broth with more variability observed. Based on this data, nutrient media composition influences the efficacy of resazurin, however, resazurin appears to be stable in aqueous solution and in bacterial growth media for up to three months. Further investigation is needed to determine what factors in the nutrient media limit the efficacy of resazurin.

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