A Novel Method to Enhance the Subcutaneous Detection of Bioluminescence in the Facultative Anaerobe, *Streptococcus pyogenes*, by DMSO-Assisted Transdermal Oxygen Delivery

Buxton DE¹, Childers BJ², Oberg KC¹,² *  
Department of Pathology and Human Anatomy¹, Surgery², Loma Linda University, Loma Linda, CA. 92350, USA  
*Email: koberg@som.llu.edu

Introduction: *S. pyogenes* is a common Gram-positive organism that can cause severe systemic and invasive disease. Xenogen developed a bioluminescent strain of *S. pyogenes* harboring the *LuxABCDE* construct, however, under anaerobic conditions, i.e. subcutaneous abscesses, the oxygen-dependent bioluminescence is reduced. To enhance subcutaneous detection of bacterial growth we developed a novel method to deliver oxygen to subcutaneous sites.

Methods: 100 ul BHI broth or blood containing 2.6 X 10⁶ *S. pyogenes* CFUs was injected subcutaneously into mice following depilation and allowed to grow for up to 24 hrs. At various time points after inoculation, the subcutaneous sites were examined for bioluminescence using the Hamamatsu Low Light Imaging System and the Metamorph imaging software. Various concentrations of DMSO and H₂O₂ separately and in combination were applied over the inoculation sites for bioluminescence detection.

Results: The administration of 30% DMSO combined with 0.6% H₂O₂ enhanced bioluminescence nearly 10 fold. The delivery of oxygen was still evident 3 hrs after DMSO/H₂O₂ administration. H₂O₂ alone (0.6%) transiently increased bioluminescence 4 fold, but appeared to be toxic as evidenced by a subsequent decrease in bioluminescence and poor response to DMSO/H₂O₂ application after further incubation (3 hrs). DMSO alone (30%) had no effect on bioluminescence detection.

Conclusions: These findings demonstrate that DMSO/H₂O₂ is a novel and useful *in vivo* approach to enhance the oxygen-dependent bioluminescence of the LuxABCDE luciferase product in an anaerobic environment. This technique will increase sensitivity in animal models allowing more precise monitoring of infection by this bioluminescent strain of *S. pyogenes*.  

This is a preprint of an article accepted for publication in Luminescence:  
Copyright 2004 John Wiley & Sons, Ltd