Use of bioluminescent *Salmonella typhimurium* DT104 to monitor uptake and intracellular survival within a human cell-line

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Clinically important bacteria, transformed with the *luxCDABE* operon, emit light as an accurate reporter of their metabolic activity. Bioluminescence as a reporter is used to monitor real-time intracellular survival of bacteria, giving a more accurate picture of microbial-host interactions that occur *in vivo* than the traditional method of viable counts. *Salmonella typhimurium* is a pathogen that colonises macrophages leading to problems in clearance and destruction. This study aimed to establish an assay using bioluminescence to investigate the uptake and intracellular survival of *S. typhimurium* in a macrophage like cell-line. *S. typhimurium* DT104 was transformed using the broad host range plasmid pBRRMCS-5 containing a Lux cassette. A photon-counting camera was used to select light emitting colonies from the gentamicin resistant transformants. The resulting recombinant bacteria were internalised within a human monocytic cell-line, THP-1. Control cultures of non-bioluminescent internalised *S. typhimurium*, non-internalised Lux+ and Lux− *S. typhimurium* and uninfected cells were also monitored. Varying multiplicities of infection and incubation periods were assessed. Extracellular bacteria were killed using colistin and the internalised location of the bacteria confirmed following the addition of saponin. The internalised bacteria gave a stable light output over 24 hours, 2 log10 higher than the negative controls, suggesting intracellular survival of the bacteria. These results indicate that this bioluminescence based assay can be used as an effective real-time method to monitor uptake and intracellular survival of *S. typhimurium* and potentially the effects of antimicrobial agents *in situ* on this clinically important pathogen.