Section of Paediatrics
Imperial College London

Room 236, Wright Fleming Institute
St. Mary's Campus
Norfolk Place
London
UK
Tel: +44 (0)20 75943191 Fax: +44 (0) 20 75943284
E-mail: s.newton@imperial.ac.uk

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Sandra Newton BSc PhD FHEA
Senior Research Fellow

FAO, Dr Jaydev Upponi (Science Editor)
Immunology and Infection
Editorial Department
JoVE
1 Alewife Center, Suite 200
Cambridge
MA 02140
USA

Dear Dr Upponi,

Further to our recent telephone and email correspondence, please find submission of the manuscript as requested titled "**Methods for the use of the invertebrate, *Galleria mellonella,* as an infection model to study the *Mycobacterium tuberculosis* complex".** We would be grateful if you would consider our manuscript for publication in the Journal of Visual Experiments.

Tuberculosis remains one of the most important human diseases worldwide. Treatment of the disease has become increasing challenging due to drug-resistant strains of *Mycobacterium tuberculosis*. There is therefore an urgent need for a greater understanding of host-*Mycobacterium tuberculosis* interactions, the development of new, anti-mycobacterial agents to target drug-resistant tuberculosis (TB) and shorten treatment regimens, and also a need for novel drug screening models that reduce and/or replace the currently used conventional animal models, all of which have limitations.

The larva of the insect, *Galleria mellonella*, has been increasingly used as a surrogate organism to study host-pathogen interactions in a range of bacterial pathogens, and as a rapid model to

screen novel antimicrobial drug candidates. In a recent study, (Li *et al*., Virulence 9:1126-37, 2018) we evaluated and established *G. mellonella* as a suitable novel infection model for the *M. tuberculosis* complex demonstrating a dose-response for *G. mellonella* survival infected with different inocula of bioluminescent, *Mycobacterium bovis* BCG *lux*, and demonstrated suppression of mycobacterial luminescence over 14 days. Through histopathological staining and transmission electron microscopy we further demonstrated that the *G. mellonella* - mycobacteria infection model can be used to study mycobacterial pathogenesis, particularly in the context of granuloma formation.

Here we describe the methods in detail for the use of *G.mellonella* larvae combined with bioluminescent mycobacteria as an infection model. *G. mellonella* has the potential to be used as a low-cost, reproducible and high-throughput, model to understand host-pathogen interactions in mycobacterial infection, and be used as a pre-screening model to assess the toxicity and activity of antimycobacterial drugs. Moreover *G. mellonella* has the capacity to significantly reduce and replace the use of animal models in TB research.

We believe these methods will be of interest to the specific community of tuberculosis, microbiologists, and infectious diseases researchers, as well as to researchers involved in drug development and discovery.

The final manuscript has been approved by all authors. The authors declare there are no conflicts of interest.

On behalf of my co-authors, I would like to thank you again for your time and kind consideration in reviewing our submission.

Yours sincerely,

S. M. Newton