A quick and easy reliable method to estimate *Anopheles stephensi* Liston (Diptera: Culicidae) pupae numbers in insectaries

CALVO, D[1].; SÁNCHEZ, J[2].; JINDAL, N[1]; LOZANO, S.[1]; HERREROS, E[1]; RODRIGUES, J[1]

[1] Malaria DPU, [2] Laboratory of Animal Sciences, Tres Cantos Medicines Development Campus, Diseases of the Developing World (DDW), GlaxoSmithkline (GSK) Tres Cantos, Spain. Email: david.8.calvo@gsk.com

Insect breeding facilities when catering to industrial level needs have to be able to meet routine demands for both, high quality and high number of robust insects whilst optimizing resources in terms of man power and time involved. In GlaxoSmithKline at Tres Cantos Medicines Development Campus (Madrid, Spain) an insectary facility has been established since 2012 as a part of a research platform dedicated to the development of new anti-malarial molecules with transmission blocking potential. *Anopheles stephensi* is reared from eggs to robust adults using a protocol that has been optimized to produce more than two thousand high quality robust females per week thereby successfully meeting the demands for mosquitoes used regularly in the Standard Membrane Feeding Assay (SMFA). It is therefore important to quickly estimate the number of pupae required in-order to provide for the corresponding number of adult mosquitoes.

There are several methods which have proved to be efficient and rapid for pupae collection from the rearing pans (Methods in Anopheles research (MR4), 2014). But when numbers matter, pupae have to be counted manually becoming a tedious, labor intensive and time consuming task. In this study we describe a new technique to quickly estimate the number of mosquito pupae collected using ImageJ software (imagej.nih.gov/ij/). Pupae were placed in a pan and the numbers per pan were first manually counted. Pupae in the pan were then photographed and the image was stored as a JPEG and pupae number were re-counted using ImageJ software. We performed a linear correlation of pupae counted manually versus those enumerated using the IMAGE J software analyzing a total of 50 pans of pupae collected from independent breeding cycles over several months. We manually estimated an average of 1934 pupae per pan and the estimated difference between the manual count and ImageJ calibration was 174.8±31.8, with a coefficient of determination of r^2=0.97. Our results demonstrate that this time saving protocol allows for a quick, accurate and reliable estimation of samples containing hundreds or thousands of pupae thereby replacing the existing manual enumeration method.