INFRAVEC2 PROTOCOL

Standardisation protocol to for Rearing Anopheles coluzzii to Maximize Plasmodium infections

Protocol Information

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PROTOCOL FOR ANOPHELES REARING TO MAXIMIZE PLASMODIUM INFECTIONS

To optimize laboratory breeding of *Anopheles coluzzii* mosquitoes for malaria research two rearing protocols from expert laboratories were compared. Differences between methods included the number of/Anopheles/larvae that were raised per tray, the available volume of water, and type of food. For "condition A" trays with 2.3 L water and ~400 larvae were prepared. These larvae were fed on day-one with Liquifry (Interpet, no. 1) and for the remaining time raised on TetraMin baby (Tetra). For "condition B" trays were filled with 700 ml water, with exactly 200 larvae. Larvae were fed custom made larval food made by taking two parts of bovine liver powder (Argentine Beef Liver Powder, NOW Foods) added to two parts of tuna meal (LT Thunfischmehl, Ref. Z30200, RSR-Baits), and one part of vitamin mix (V1007-100G, Sigma Aldrich). This dry powder was thoroughly mixed and stored at 4°C, and used to prepare liquid food by dissolving 2% powder in deionized water. Following dosing optimization, a starting batch of eggs was divided into two groups (parental generation). To compare the two protocols, three generations were raised simultaneously, and potential variations between both methods were identified by assessment of the mosquito's body size, by three indicators. The imbibed blood-meal volume after membrane feeding was determined, oocyst intensity and infection prevalence was evaluated (D8 PI of/P. falciparum/gametocyte blood-meal) and wing length was measured. Small differences between both conditions for blood-meal volume and wing length were observed, with mosquitoes raised by condition A to be larger in F2 and F3 but variation in body size being smaller in condition B. When comparing infection intensity and prevalence, no differences were observed, indicating that comparable transmission efficiency was achieved. Findings and protocols will be presented in more detail at a later stage.

