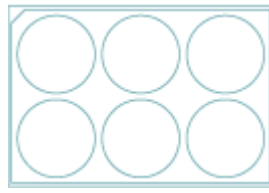


0.3-1% Asexual stage culture at 4% HCT



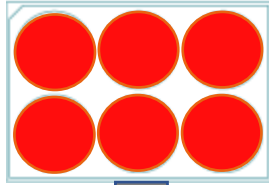
+



Day 0



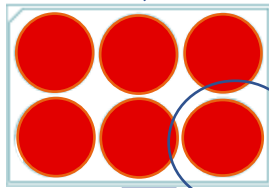
Add 5 ml of parasite culture to each well



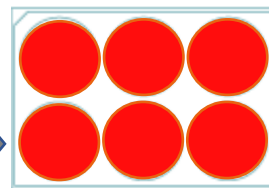
Day 1-3



Daily media change (leave 1-2ml supernatant to avoid removal of cells and add 5ml of fresh complete media)



Day 3

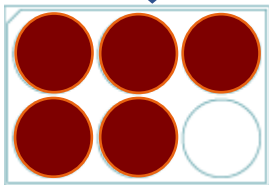


Split one well to either maintain asexual stage or initiate another gametocyte culture

Day 4-15



Daily media change



Make blood smear and calculate mature gametocytemia

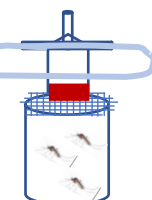
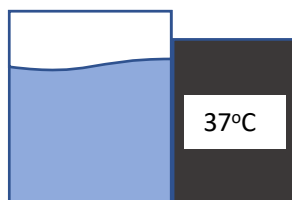


Days 15-18

Transfer culture to pre-warmed centrifuge tube and spin at 500g for 5min



Dilute gametocyte culture pellet to desired concentration (0.02-0.3%) with prewarmed reconstituted blood.



Pipet 200-1000 μ l blood feed into prewarmed glass feeder and feed for 30min

Discard unfed mosquitoes, transfer fed mosquitoes to 26°C incubator and place 10% sugar-soaked cotton pads on top of the mosquito cage



Days 7-8 Post feeding

Dissect mosquito midgut, stain with 0.2% Mercurochrome and count oocysts on microscope at 10x objective

Days 14-21 post feeding

Dissect mosquito salivary gland for sporozoites isolation