Large Scale Production of Late Stage *Plasmodium falciparum* Gametocytes.

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**INTRODUCTION**

With the drive to eradicate malaria comes the requirement for more research into the transmission stages of *Plasmodium falciparum* (Pf). Research on this stage of the parasite life cycle is expanding in all areas of biology and biochemistry including compound screening, proteomics, genomics and vaccine development. During Pf malarial infection, some parasites switch from assexual reproduction and develop into erythrocyte resident gametocytes, also known as the sexual stages. Pf gametocytes progress through five developmental stages (I-V), over 10-12 days, with mature stage V forms being transmissible to mosquitoes. Although researchers have been successfully culturing gametocytes in vitro for decades, recent technological advancements have necessitated not only large scale production but also high synchronicity and greater purity. This poster describes the protocol used to produce highly synchronous late stage gametocyte cultures in litre quantities, without the requirement of specialised laboratory equipment.

**SEXUAL CULTURE PROGRAM**

Asexual cultures with less than 2 months total time in culture must be used. Daily, small splits of the culture to minimise stress related spontaneous gametocyte production. Sorbitol synchronisation of ring predominant culture performed at least 96 hours before GAM induction. Incremental culture volume increase over 4-8 days depending on the volume of culture required with Trophozoite/Schizont predominant culture harvested and mature parasites isolated using MACs column technology (magnetic cell sorting/isolation system).

**PARASITE STRESS FOR GAMETO CYTE INDUCTION**

Stress of the resulting ring stage parasite is performed using a conditioned media to fresh media mix. The ratio of Fresh to conditioned media is dependent on % parasitemia obtained after overnight shaking. The culture is split to 12% rings and the hematocrit increased to 2.5% by the reduction in culture media volume. The parasites are incubated in static culture overnight. For ease of access the culture is maintained in large petri dishes at 50ml per dish. 35ml of media/dish is removed first thing in the morning and replaced with 35ml of fresh media. Mid morning to early afternoon the resulting schizonts are split to 3% parasitemia and the resulting culture poured into cell factories which are gassed (reduced O2) and placed on a shaker overnight.

**CONCLUSION**

This optimised protocol can be used to produce $10^8$ gametocytes per induction which are predominantly from the same generation of assexual parasites converting within the same cycle. Some younger gametocytes do occur from minor assexual parasite contamination in the induced gametocyte culture but this is minor. A bonus of this protocol, is the isolation of less synchronised gametocytes which are obtained at high parasitemia but low hematocrit and volume. The only equipment required for this large scale production of synchronous gametocytes is an incubator with O2 reduction capacity, Cell factories, a mixed gas cylinder and regulator and a MAC column separator and columns. The limiting factor for this work is the MAC separators. The more you have the larger the cultures that can be handled and the greater the gametocyte yields.