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

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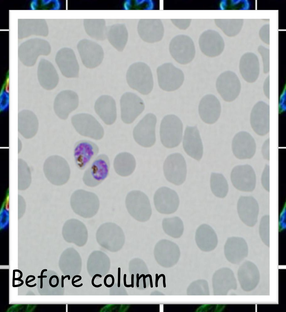
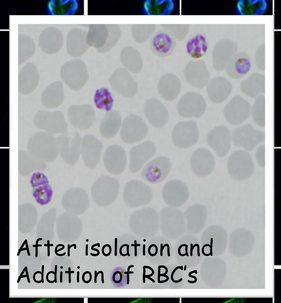
INTRODUCTION

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 asexual 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.

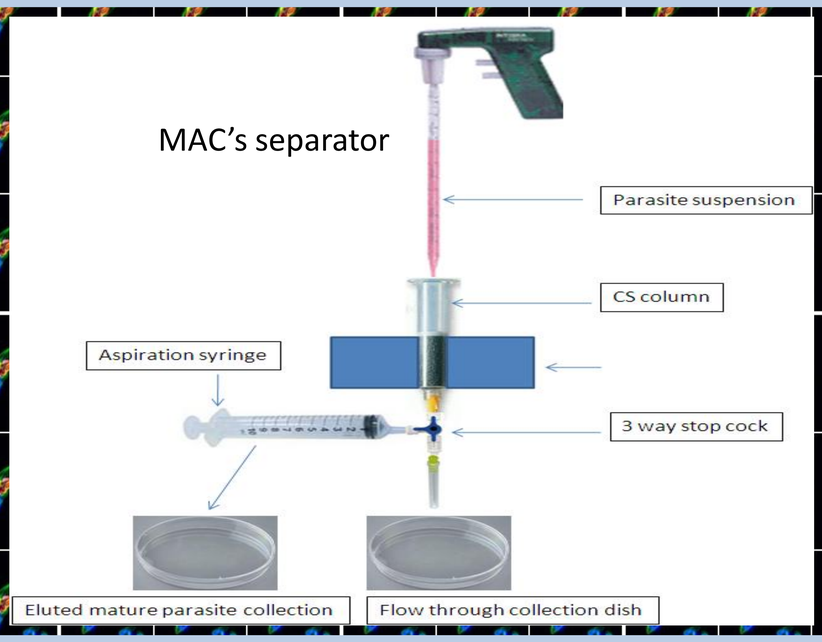
A SEXUAL CULTURE PROGRAM

Asexual cultures with less than 2 months total time in culture must be used. Daily, small splits of the culture to minimise stress related spntaneous gametocyte production. Sorbitol syncronisation 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 MAC's column technology (magnetic cell sorting/isolation system)


Before column


After isolation and Addition of RBC's

Increase % parasitemia for induction without overly stressing parasites. No dramatic drops in Parasitemia throughout Culture until induction Instigated.



Isolated Trophozoite/schizonts put under Gametocyte inducing conditions : Human serum/albumx mixture, 3% trophozoites and 1.25% Hematocrit. Overnight shaking in a reduced oxygen gassed cell factory.

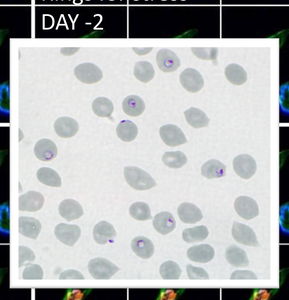
Up to 800ml per 4 tier cell factory

PARASITE STRESS FOR GAMETOCYTE INDUCTION

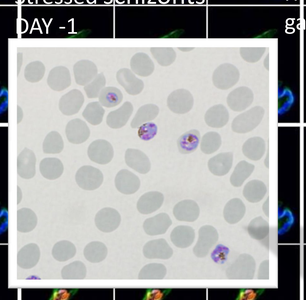
Stress of the resulting ring stage parasite is performed using a conditioned media to fresh media mix. The ratio of Fresh to conditioned media is dependent on % parasitemia obtained after overnight shaking. The culture is split to 12% rings and the hematocrit increased to 2.5% by the reduction in culture 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.

Hemozoin containing GAMs and schizonts/trophozoites are emoved by MACs separation on DAY 0. The isolated hemozoin containing parasites are kept and exchanged into NAG (N-acetyl-glucosamine) media with the addition of none infected RBC's.The Remaining ring morphology parasites are exchanged into NAG media for removal of Asexual parasites. The cultures are maintained in 50ml volumes at 2.5%H in large petri dishes. This allows for easy media exchange on a daily basis for 8 days before isolation on MAC columns

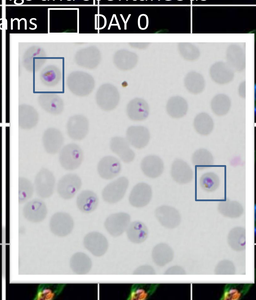
Rings for stress DAY -2



Stressed schizonts DAY -1

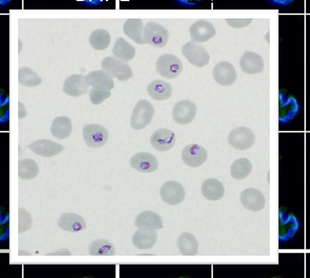


rings and spontaneous gams DAY 0

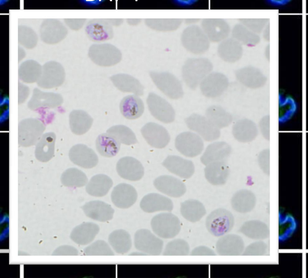


A

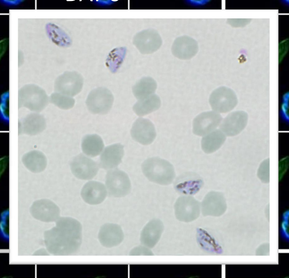
DAY 1



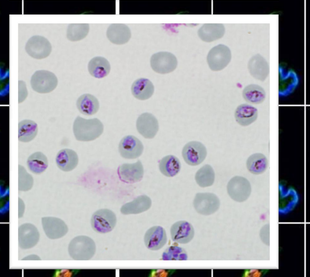
DAY 4

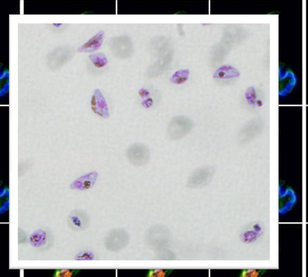


DAY 6



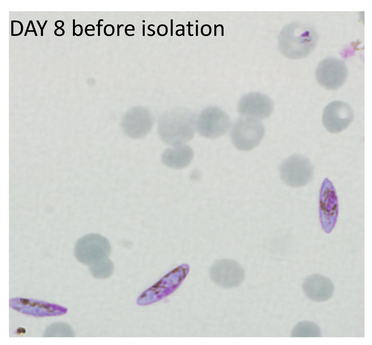
B



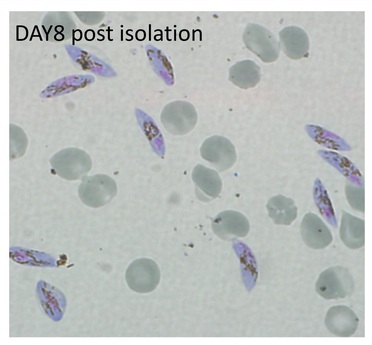


A. Synchronous gametocyte culture from one generation
B. B. spontaneously generated none synchronous isolated
C. Gametocytes at day 0

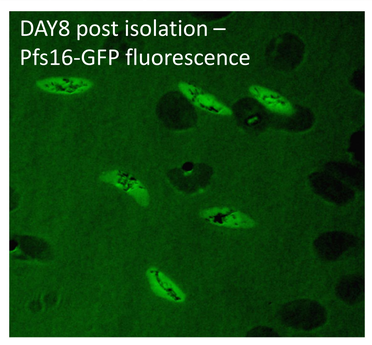
DAY 8 before isolation



DAY8 post isolation



DAY8 post isolation – Pfs16-GFP fluorescence



Comparison of two different transgenic NF54 –GFP cultures and the effect of time in culture

parasite culture	Volume of culture	% trophs of starting material	Volume of culture at DAY 0	Parasite/ml(10^6)	Total # of parasites 10^6	Age of parasite at induction
pfs16-N2 S2 1	100ml	2.33	200	13.635	272.70	2 weeks
pfs16-N2 S2 2	100ml	1.78	120	10.122	202.44	
pfs-48/45-2A	100ml	1.35	150	3.167	31.67	>2.5 months
pfs-48/45-2B	100ml	1.33	120	1.8	18.00	
pfs16-NB-2	100ml	1.85	80	2.625	26.25	>2.5 months

Gametocyte yields drop dramatically over time spent in continuous culture. This drop is approximately 10 fold over the space of the 2-3 months spent in culture.
We have the capacity to isolate from 2L of asexual culture and, therefore, produce > 4,000,000,000 synchronised late stage gametocytes. Ie 4 x 10^9 gametocytes per large scale screening run when using cultures which have been in culture for only a couple of weeks. Using older cultures reduces the capacity 10 fold.

CONCLUSION

This ***optimised*** protocol can be used to produce 10^9 gametocytes per induction which are predominantly from the same generation of asexual parasites converting within the same cycle. Some younger gametocytes do occur from minor asexual 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.