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Automated Synchronization of *P. falciparum* using a Temperature Cycling Incubator

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Abstract

As malaria keeps affecting millions of lives every year, research based in culture of *Plasmodium falciparum in vitro* needs to be efficient and accurate. The development of better techniques and methodologies for the growth and maintenance of the parasites can save money, time, and lead to more trustable results. It has been observed, first in patients and then in the laboratory, that the malaria *falciparum* parasites growth is affected by high temperatures. This trait can be used with laboratory cultures to synchronize and maintain the parasites in the same stage of their cell cycle. This harmony of stages is very desirable for the purpose of conducting metabolomic, proteomic and transcriptome analysis as well as for drug screening. Most scientists in the field of malaria use chemicals (usually sorbitol) that kill certain stages of the parasite to obtain synchronization, but this latter method does not last long and the parasites thus treated should not be used for assays immediately after the treatment, due to the toxic effects that might have been infringed in the culture. A temperature cycling incubator (TCI) was acquired in our laboratory and it was used to test the synchronization of the multidrug resistant W2 and chloroquine resistant 7G8 strains, commonly used in our bioassays where they and their synchronization constitute essential tools for our drug discovery program. We followed the protocol designed by Haynes and Moch in 2002 and we made a comparison of the effectiveness of each of the two methods, chemical and temperature based. Our results show W2 synchronization by temperature cycling, with the help of an initial use of 0.3 M alanine, to last more than two months while a tight synchronization with the use of 5% sorbitol was lost as rapidly as in one week. Sorbitol could also be used with the TCI for synchronization with good results. However, 7G8 could not be efficiently synchronized with temperature cycling using the same program as that of W2.

Key Words: *P. falciparum*, Culture, Synchronization, High temperature, TCI.

Introduction

It has been described that *in vitro* growth of *Plasmodium falciparum* is inhibited by high temperatures (1-5) and there have been relationships drawn from these findings on how fever in malaria patients may influence parasite growth, coordination of cyclic stages, and parasitemia.

In 2002, Haynes and Moch published a method for using these observations to synchronize *P. falciparum* cultures for laboratory

use (6). For almost every *in vitro* experiment with this parasite there is a need for a synchronized culture. Traditionally, a chemical like sorbitol has been used to kill the mid to late stages of *P. falciparum*, leaving only rings alive and thus synchronizing the culture. The method optimized by Haynes and Moch saved the parasite from constant exposure to a foreign chemical and made maintenance of culture more amenable to the labor hours of technicians and scientists. The method used an exact cycle of 48 hours for the completion of the cell cycle. They used the FVO, Dd2, and 3D7 strains to test their system and gave suggestions on other strains that could be synchronized through temperature (FCR-3, CAMP, and FCB-2). We incorporated their temperature cycling methodology to try to synchronize the 7G8 (7) strain and the chloroquine resistant strain, W2 (8) used in our bioassays for drug screens; here we report the conditions used and the outcome of our test.

Material and Methods

Culture: We cultured strains 7G8 and W2 using the conventional method of Trager & Jensen (9) with some modifications, that include the use of RPMI 1640 medium modified (Sigma-Aldrich), 25 mM HEPES, 15 µM hipoxanthine, 50 mg/mL gentamicine sulfate, and 200 mM L-Glutamine, supplemented with 10% human serum, 2% sodium bicarbonate and a balanced mix of gases (90% N_2, 5% O_2 and 5% CO_2). Cultures were checked every day for optimal development and health by making thin film slides stained with Giemsa and having their parasitemia assessed by light microscopy for up to 70 days.

Synchronization of parasites: Cultures of 7G8 and W2 were incubated in parallel in a temperature cycling incubator (TCI) (Cooled Incubator, Sanyo**,** Model MIR-154) and a static temperature incubator set at 37°C (Lab-Line Instruments Inc. Imperial III Incubator). The TCI temperature fluctuations and their exposition times are as following: (i19:30), 22°C (2:22), 37°C (13:42), 39.8°C (9:30), 37.2°C (21:26), 38.2°C (1:00) (same as Haynes´conditions for FVO); making the parasite cycle last exactly 48 hours. We used 0.3 M alanine (Sigma-Aldrich) as first treatment for synchronization of cultures in TCI (5) and 5% sorbitol for cultures in a 37°C incubator, as described (10). We made a comparison between the collected data from the W_2 and 7G8 strain culture of *P. falciparum* exposed to the febrile temperatures of the TCI and those set at 37°C in a normal incubator.

Results and Discussion

The TCI method synchronized our culture obtaining values between 85% and 100% synchrony during a 70 day period (Figure 1). At this point we stopped counting. It is worth noting that with this method we can also obtain high parasitemias if needed. In the cultures kept at 37°C and treated with sorbitol, as used to maintain

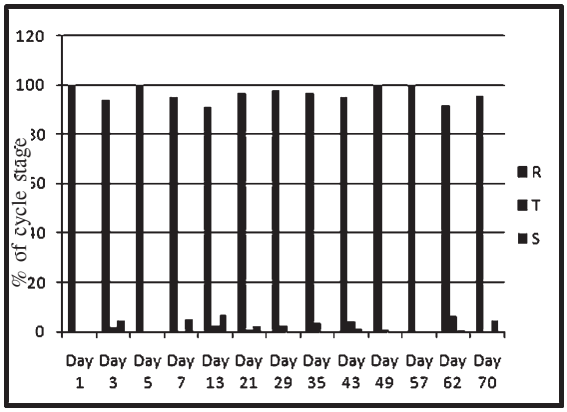

Fig. 1. Synchronization of *P. falciparum* by alanine and incubation in a TCI. To asses synchronization the percentage of each of the stages of the cell cycle of the parasite was determined in Giemsa stained smears taken from the culture on the days depicted in the graph. Parasites were treated one time with 0.3M alanine at the beginning of the culture and then they were placed on a temperature cycling incubator (TCI). R (rings), T (trophozoites), S (schizonts).

our parasites, we only got synchronization for a little over a week (Figure 2), and almost never observed 100% synchronization. While at the first day after treatment there were 93.8% of rings, by day 9 there was already a mix of 43.3% rings, 36.7% trophozoites, and 20% schizonts.

For the purpose of comparing if the choice of chemical used at the beginning of the synchronization period was responsible for the success of the method, we placed a culture synchronized with sorbitol into the TCI. Although alanine proved to be slightly superior, excellent synchronization results were again obtained with sorbitol, which points out to the great advantage of using a TCI over a 37°C incubator, and that this is the major factor responsible for the good outcome of synchronization numbers. One thing to note, though, is that at constant 37°C we always got higher parasitemias than in the TCI incubator, but the culture was less synchronized.

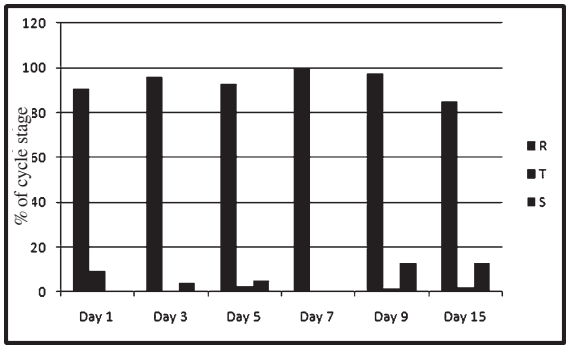

Fig. 2. Synchronization of *P. falciparum* by sorbitol and use of a 37°C incubator. To asses synchronization the percentage of each of the stages of the cell cycle of the parasite was determined in Giemsa stained smears taken from the culture on the days depicted in the graph. Parasites were treated one time with 5% sorbitol at the beginning of the culture and then they were placed on a 37°C constant temperature incubator. R (rings), T (trophozoites), S (schizonts).

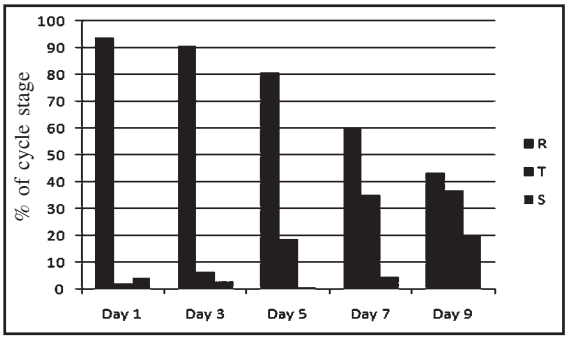

Fig. 3. Synchronization of *P. falciparum* by sorbitol and use of a TCI. To asses synchronization the percentage of each of the stages of the cell cycle of the parasite was determined in Giemsa stained smears taken from the culture on the days depicted in the graph. Parasites were treated one time with 5% sorbitol at the beginning of the culture and then they were placed on a temperature cycling incubator (TCI). R (rings), T (trophozoites), S (schizonts).

As for the 7G8 strain, with the alanine treatment and incubation in the TCI, we could not obtain the synchronization last for more than a week with the temperature program we used for the W2 strain (data not shown). After their publication in 2002 where they reported problems synchronizing 7G8 with their TCI method, Moch and Haynes have been able to put 7G8 in synchrony using this methodology, with a different temperature program (personal communication). For a malaria research laboratory, if it is possible to acquire more than one temperature cycling incubator, it is worth the investment. Together with the alanine treatment which seems to be less toxic on the parasites than the sorbitol one (11), the time shifts of technicians are perfectly adjusted to when they receive samples for the bioassays, making the method highly efficient and work amenable.

Conclusions

The repetitive automated synchronization cycle achieved with TCI makes availability of parasites within work hour schedules, at the same time that it helps satisfying specific aims of our research that require high synchronization. Through adjustments in programming of the TCI, different strains of *P. falciparum* can be highly synchronized, considering that each one has different cycles of temperatures and times of

exposure. In our case, the W2, a highly valuable strain for drug screening, can be satisfactorily synchronized with this methodology while retaining great viability and making the results of bioassays more accurate.

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References

1. Aunpad, R., Somsri, S., Na-Bangchang, K., Udomsangpetch, R., Mungthin, M., Adisakwattana, P. and Chaijaroenkul, W. (1998). The effect of mimicking febrile temperature and drug stress on malarial development. Annals of Clinical Microbiology and Antimicrobials, 8: 19.

2. Long, H.Y., Lell, B., Dietz, K., Kremsner, P.G. (2001). *Plasmodium falciparum: in vitro* growth inhibition by febrile temperatures. Parasitol Res, 87 (7): 553-555.

3. Kwiatkowski, D. (1989). Febrile temperatures can synchronize the growth of *Plasmodium falciparum in vitro*. J Exp Med, 169 (1): 357-61.

4. Gravenor, M.B., Kwiatkowski, D. (1998). An analysis of the temperature effects of fever on the intra-host population dynamics of *Plasmodium falciparum*. Parasitology, 117 (Pt 2): 97-105.

5. Pavithra, S.R., Banumathy, G., Joy, O., Singh, V., Tatu, U. (2004). Recurrent fever promotes *Plasmodium falciparum* development in human erythrocytes. J Biol Chem, 279 (45): 46692-46699.

6. Haynes, J.D., Moch, J.K. (2002). Automated Synchronization of *Plasmodium falciparum* Parasites by Culture in a Temperature-Cycling Incubator. Methods in Molecular Medicine, 72: 489-497. Humana Press, Inc., Totowa, NJ

7. Bhattacharjee, AK., Kyle, DE. and Vennerstrom, JL. (2001). Structural Analysis of Chloroquine Resistance Reversal by Imipramine Analogs. Antimicrobial Agents and Chemotherapy, 45: 2655-2657

8. Burkot, T.R., Williams, J.L., Schneider, I. (1984). Infectivity to mosquitoes of *Plasmodium falciparum* clones grown in vitro from the same isolate. Trans R Soc Trop Med Hyg, 78:339-341.

9. Trager, W., Jensen, J.B. (1976). Human malaria parasites in Continuous culture. Science, 193: 673-675.

10. Lambers, C. and Vanderberg, J. (1979). Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. J Parasitol, 65 (3): 418-420.

11. Dagan, A., Efron, L., Gaidukov, L., Mor, A., and Ginsburg, H. (2202). In Vitro Antiplasmodium Effects of Dermaseptin S4 Derivatives. Antimicrobial Agents and Chemotherapy, 46: 1059-1066.