Published in final edited form as:

Exp Parasitol. 2014 May ; 140: 18–23. doi:10.1016/j.exppara.2014.03.010.

Refrigeration provides a simple means to synchronize *in vitro* cultures of *Plasmodium falciparum*

Lili Yuan^{1,§}, Mingming Hao^{1,†,§}, Lanou Wu^{2,§}, Zhen Zhao^{1,§,2}, Benjamin M. Rosenthal³, Xiaomei Li⁴, Yongshu He⁶, Ling Sun¹, Guohua Feng⁵, Zheng Xiang¹, Liwang Cui^{7,*}, and Zhaoqing Yang^{2,*}

¹Department of Pathogen Biology and Immunology, Kunming Medical University, Yunnan Province 650500, China

²Department of Pharmacology, Kunming Medical University, Yunnan Province 650500, China

³Animal Parasitic Disease Laboratory, Agricultural Research Service, US Department of Agriculture, BARC East Building, 1180 Beltsville, MD 20705, USA

⁴School of Public Health, Kunming Medical University, Yunnan Province 650500, China

⁵Center for Biomedical Engineering Research, Kunming Medical University, Yunnan Province 650500, China

⁶Department of Cell Biology and Genetics, Kunming Medical University, Yunnan Province 650500, China

⁷Department of Entomology, The Pennsylvania State University, 501 ASI Bldg., University Park, Pennsylvania 16802, USA

Abstract

Plasmodium falciparum is usually asynchronous during *in vitro* culture. Highly synchronized cultures of *Plasmodium falciparum* are routinely used in malaria research. Here, we describe a simple synchronization procedure for *P. falciparum* asexual erythrocytic culture, which involves storage at 4°C for 8–24 h followed by routine culture. When cultures with 27–60% of ring stage were synchronized using this procedure, 70–93% ring stages were obtained after 48 h of culture and relative growth synchrony remained for at least two erythrocytic cycles. To test the suitability of this procedure for subsequent work, drug sensitivity assays were performed using four laboratory strains and four freshly adapted clinical *P. falciparum* isolates. Parasites synchronized by sorbitol treatment or refrigeration showed similar dose-response curves and comparable IC₅₀

© 2014 Elsevier Inc. All rights reserved.

*Corresponding authors: Liwang Cui: Department of Entomology, The Pennsylvania State University, 501 ASI Bldg., University Park, PA 16802. Phone: (814) 863-7663; Fax: (814) 865-3048; luc2@psu.edu. Zhaoqing Yang: Department of Pathogen Biology and Immunology, Kunming Medical University, 1168 West Chunrong Road, Kunming, Yunnan Province 650500, China. Phone: 86-871-8225541. zhaoqingy@yahoo.com.

†Current Address: Dali State Schisto Control Research Institute, Dali, Yunnan, China

§These authors contributed equally.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

values to four antimalarial drugs. The refrigeration synchronization method is simple, inexpensive, time-saving, and should be especially useful when large numbers of *P. falciparum* culture are handled.

Keywords

Synchronization; *Plasmodium falciparum*; Refrigeration; Drug susceptibility

1. Introduction

Malaria continues to be a leading cause of morbidity and mortality worldwide. It is responsible for more than 250 million cases annually, resulting in almost one million deaths. Four *Plasmodium* species (*P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*) cause human malaria and the monkey parasite *P. knowlesi* has emerged recently as a threat to cause zoonotic malaria in human populations of Southeast Asia (Collins, 2012). Malaria parasite development in human hosts is highly synchronous. Synchronous rupture of intraerythrocytic schizonts and the appearance of their progeny in the circulating blood are associated with the most unique clinical feature of malaria – paroxysmal fever.

The development of *in vitro* culture technique of the human malaria parasite *P. falciparum* has enabled studies of many biological aspects of this parasite (Trager and Jensen, 1976). During *in vitro* culture, *P. falciparum* development is usually asynchronous with all asexual stages of the parasite present at any given time. The generation of cultures containing highly synchronized parasites is necessary for various studies. Various synchronization methods have been established, which rely on removal of different parasite stages by differential osmotic lysis or chemical poison (Lambros and Vanderberg, 1979, Pfaller et al., 1982), physical separation relying on differential density (Aley et al., 1984, Jensen, 1978, Mrema et al., 1982), magnetic separation (Ahn et al., 2008, Bhakdi et al., 2010, Heidrich et al., 1982, Paul et al., 1981), or cell cycle or DNA polymerase inhibitors (Assaraf et al., 1986, Heidrich, et al., 1982, Hensmann and Kwiatkowski, 2001, Hoppe et al., 1991, Hui et al., 1983, Inselburg and Banyal, 1984, Lelievre et al., 2005, Naughton and Bell, 2007, Scragg et al., 1999). Sometimes, two of the these methods are combined to achieve higher levels of synchronization and a narrow window of parasite development (Inselburg, 1983, Jensen, 1978, Lelievre et al., 2005, Pasvol et al., 1978, Radfar et al., 2009, Ranford-Cartwright et al., 2010, Spadafora et al., 2011). Temperature cycling has also been reported as a means to improve synchronization (Haynes and Moch, 2002). This method achieves synchronization by cycling the temperature between 17–40°C in several complicated steps.

Here we report a novel physical synchronization method of *P. falciparum in vitro* culture based on differential sensitivity of different asexual erythrocytic stages to refrigeration, which can yield 70–93% ring stage parasites. The method is simple to perform, requires no specialized equipments or reagents, and is suitable for *in vitro* drug sensitivity assay.

2. Materials and methods

2.1. Parasites and *in vitro* culture

P. falciparum laboratory strains 3D7, HB3, DD2, and 7G8 obtained from the Research and Reference Reagent Resource Center (MR4) (Manassas, VA) four clinical isolates WB299, WB548, WB183 and WB682 collected from the China-Myanmar border area were cultured and used for *in vitro* drug assay. Parasites were routinely cultured in fresh type O human red blood cells (RBCs) at a 5% hematocrit in a complete medium [RPMI 1640 (10.4 g/liter), HEPES (5.94 g/L), hypoxanthine (50 mg/L), Albumax II (5 g/L), gentamicin (50 mg/L), and NaHCO₃ (2.1 g/L)] at 37°C in 25 cm² flasks (Costar) under a gas environment of 92% N₂, 5% CO₂, and 3% O₂. Synchronization of parasite cultures with 5% sorbitol (Sigma) was performed as previously described (Lambros and Vanderberg, 1979).

2.2. Parasite synchronization by refrigeration

To determine the effect of temperature and treatment time on parasite development, cultures of the *P. falciparum* 3D7 clone at 40–50% of ring stage were stored at either 4°C or 8°C for varying intervals (4, 6, 8, 10, and 24 h). After the refrigeration treatment, cultures were removed and continuously cultured at 37°C for 48 h. These cultures were examined at 24, 48, 72 and 96 h by microscopy using Giemsa-stained smears to determine parasitemia and proportion of different developmental stages of (rings, trophozoites, and schizonts). Using defined cooling conditions (4°C for 24 h), we further tested whether this method is suitable for synchronizing cultures at different proportions of ring-stage parasites (27–44% or 45–60%). For each treatment, 2–3 biological replications were tested. In subsequent experiments, cultures with >30% ring stage parasite were synchronized by refrigeration at 4°C for ~24 h.

2.3. The effect of refrigeration on schizont-stage parasites

To purify schizont-stage parasites, synchronized 3D7 parasite culture at schizont stage were loaded on a 40 – 70% Percoll step gradient. After centrifugation, schizonts were collected from the 40/70 interface. Purified schizonts were diluted to 0.5% parasitemia with fresh RBCs, and divided into two flasks. One flask was stored under 4°C for 20 h followed by culturing at 37°C for 36 h. The other flask was used as control and cultured at 37°C for 36 h without subjecting to the refrigeration treatment. Smears were made from these cultures, stained with Giemsa, and examined by microscopy.

2.4. Comparison of refrigeration and sorbitol treatment methods for synchronization

To compare the synchronization efficiency of the refrigeration and sorbitol treatment methods, cultures of 3D7 parasites were divided into two aliquots. One aliquot was subject to 5% sorbitol treatment, whereas the other was stored at 4°C for 24 h. Both treatments were followed by culturing under standard conditions for 48 h. Parasite stages were differentiated and counted in Giemsa-stained thin smears. A total of five cultures with different proportions (30–50%) of initial rings were compared between the two synchronization methods.

2.5. Drug assay

To determine the suitability of the refrigeration synchronization method for subsequent drug assays, parasites from four laboratory strains and four field isolates were assayed in parallel for sensitivity to dihydroartemisinin (DHA) (Kunming Pharmaceutical Co., Kunming, Yunnan, China), Piperaquine (PPQ) (Chongqing Kangle Pharmaceutical Co. Ltd.), chloroquine (CQ), and quinine (QN) (Sigma). The stock solutions of CQ (3.0 mM) and PPQ (0.3 mM) were prepared in distilled water and methanol, respectively. The stocks of QN (25.6 μ M) and DHA (0.3 mM) were made in ethanol. Parasites from the same cultures were divided into aliquots and synchronized by either sorbitol treatment or refrigeration at 4°C for 24 h. Each synchronized parasite culture at the ring stage was diluted with complete medium and fresh RBCs to a 2% hematocrit and 1% parasitemia. Parasite sensitivities to the four drugs were determined using a SYBR green I-based fluorescence assay (Meng et al., 2010, Smilkstein et al., 2004). For drug assays, 80 μ l of the parasite suspension were dispensed into the test wells of a 96-well microtiter plate, to which 20 μ l of DHA, CQ, PPQ and QN solution was added to each well. Serial dilutions of each drug were made in a complete medium to obtain the desired final concentrations for CQ (0, 1, 4, 16, 64, 256, 1024, and 4096 nM), PPQ (0, 1, 3, 9, 27, 81, 243, and 729 nM), QN (0, 10, 40, 160, 320, 640, 1280, and 2560 nM), and DHA (0, 0.05, 0.15, 0.5, 1.5, 5.0, 15, 50, 150, 500, and 1500 nM). For each drug concentration, three replicates were performed. In addition, two biological replicates of the drug assay were performed on separate days. The drug concentration that inhibits 50% of the growth of the parasites (IC_{50}) was calculated by using GraphPad Prism.

2.6. Statistical analysis

The proportions of ring stage from different treatment groups were analyzed by the Wilcoxon W Test. The IC_{50} of different strains between the two synchronization methods was analyzed by Paired Hotelling's T^2 Test.

3.1. Results

3.1. Culture synchronization by refrigeration: temperature and time comparisons

Since ring stage *P. falciparum* can tolerate treatment at lower temperatures, we wanted to determine whether refrigeration kills other stages to achieve synchronization of the culture. Cultures of 3D7 parasites at 40–50% ring stage were divided into groups and stored at either 8°C or 4°C for 4, 6, 8, 10, and 24 h. All parasite groups stored at 8°C and 4°C for 4 – 6 h resulted in parasite cultures with < 70% ring stage after 48 h of culture, whereas the groups stored at 4°C for 8 – 24 h had > 70% ring-stage parasites (Table 1).

To determine whether the initial proportions of rings affect the efficiency of synchronization, parasite cultures with different proportions of rings were divided into two groups and treated at 4°C for ~24 h. After refrigeration synchronization, the parasite group with 27–44% initial rings achieved 70–93% synchrony (median 76, N= 10), whereas parasite group with 45–60% initial rings was synchronized to 73–91% (median = 83, N=10). Although the group with higher initial proportions of rings had higher levels of synchrony after the treatment, the difference between the two groups were not statistically different

(Wilcoxon W Test, $Z=-0.719$, $P=0.472$), suggesting that this method can be used in cultures with different proportions of starting ring-stage parasites.

To further test whether parasites synchronized by refrigeration could remain synchronized for two intraerythrocytic cycles, an asynchronous 3D7 culture with 43% rings, 37% trophozoites, and 20% schizonts was treated at 4°C for 12 h (Fig. 1). At 48 h, the parasite culture was highly synchronous with ~92% of the parasites at the ring stage (Fig. 1). After two erythrocytic cycles, the culture was still relatively synchronized with ~80% at the ring stage, suggesting that parasite growth could remain synchronous for at least two intraerythrocytic cycles. This result also showed that the ring stage parasites were transiently arrested by refrigeration, and they resumed normal growth after being returned to the standard culture conditions.

3.2. Sensitivity of schizonts to refrigeration

To test whether schizonts are sensitive to refrigeration, we purified schizonts and incubated them at 4°C for 20 h followed by 36 h of culture. Without the refrigeration treatment, the parasites grew to late trophozoite stage and reached ~1% parasitemia. However, in the refrigeration treated group, no parasite growth was detected, suggesting that schizont development or/and merozoites invasion process was blocked by cold treatment.

3.3. Comparison of synchronization methods

We then compared the synchronization efficiency of the refrigeration method and the standard sorbitol method using 3D7 cultures at different initial ring ratios (30–50%). Refrigeration treatment at 4°C for 24 h and subsequent culture for 48 h achieved >70% ring stage (median 82.4%, range 70–93%). One round of 5% sorbitol treatment also resulted in >70% of ring stages (median 75.7%, range 70–94%). There was no significant difference in the ring percentage between the two methods (Wilcoxon Test, $Z=-0.105$, $P=0.917$). To further compare growth curves of cultures synchronized by these two methods, we used a 3D7 culture with ~43% rings. After synchronization of two culture aliquots by either sorbitol treatment or 4°C refrigeration for 12 h, the resulting ring-stage parasites were used to seed cultures at 0.5% parasitemia and their growth was monitored for two intraerythrocytic cycles. The results showed that daily parasitemias of the two cultures were not significantly different ($P>0.05$), albeit parasitemias of the 4°C synchronized culture at 48 and 72 h were slightly lower than those from the sorbitol-treated culture (Fig. 2).

3.4. *In vitro* drug assay with synchronized parasites

Parasites from four laboratory strains and ten field isolates were synchronized by both sorbitol treatment and refrigeration methods, and the synchronized ring-stage parasites were used in a standard drug assays using the SYBR Green I method. For each of the four drugs (DHA, QN, CQ and PPQ), there is no significant difference in the drug response curves Z (Fig. 3) or IC_{50} values (Table 2) between the two synchronization methods (Paired Hotelling T^2 Test, $P>0.05$). This result indicated that the refrigeration synchronization method can be applied to *in vitro* drug assays.

4. Discussion

Since *P. falciparum* is usually asynchronous during *in vitro* culture, the generation of highly synchronized parasite cultures is necessary for studies of various aspects of parasite biology such as cell cycle, drug susceptibility, dynamic global gene expression during development (Radfar et al., 2009, Ranford-Cartwright et al., 2010). For *in vitro* drug assays, >70% ring stage are normally required. The most used method is the 5% sorbitol treatment of the culture when the majority of the parasites are at the ring stage. Sorbitol treatment only kills the trophozoites; it is difficult to achieve a narrow window of parasite development. Thus, two rounds of synchronization are usually performed in order to achieve >90% synchrony. Although seemingly simple and only requiring centrifugations, sorbitol treatment is still time consuming and requires at least 20 min to complete its 12 procedural steps for each culture flask. In addition, such a manual manipulation also introduces a higher risk of culture contamination.

Based on the fact that only *P. falciparum* ring-stage parasites appear to survive substantially lower temperatures and are the stage for cryopreservation, we designed a simple cooling method for synchronizing *P. falciparum* cultures. It is known that RBCs can be maintained for 35 days at $4 \pm 2^\circ\text{C}$. We have shown that relatively high levels of synchronization (>70%) can be achieved by 4°C storage for 8 – 24 h, and the synchronized parasites are suitable for *in vitro* drug assays. Although the starting cultures are preferably at a higher proportion of rings, we found that this cooling method can be used with cultures at 27– 60% rings. This approach to synchronization is easy, inexpensive, and labor-saving. It is especially conducive in settings requiring the processing of many samples in a short time period. In addition to the cooling regimen of 4°C for 24 h, we found that storage at 4°C for 8 – 48 h achieved similarly high levels of culture synchronization (data not shown). This means that the initial cultures can be conveniently synchronized by storing in a 4°C refrigerator for 14 – 24 h, and downstream work with the synchronized cultures can be easily performed on the next day. It is noteworthy that this physical synchronization method by refrigeration does not require treatment of the parasites with chemical reagents, which might affect subsequent assays. In the future, it would be desirable to understand whether refrigeration offers benefits as a synchronization method for other kinds of downstream procedures or research methodologies.

Acknowledgments

We thank Dr. Jun Miao for technical advice. This project was funded by the National Institute of Health (U19 AI089672 to LC, 1R01AI075429 to ZY), National Science Foundation of China (No. U1202226, 81161120421 and No. 31260508 to ZY), the Doctor Science Research Foundation of the Education Ministry of China (No. 20125317110001 to ZY) and Yunnan Science Foundation (No. 2012FB153 to ZX).

Abbreviations

DHA	dihydroartemisinin
PPQ	piperaquine
CQ	chloroquine

QN quinine

References

- Ahn SY, Shin MY, Kim YA, Yoo JA, Kwak DH, Jung YJ, Jun G, Ryu SH, Yeom JS, Ahn JY, Chai JY, Park JW. Magnetic separation: a highly effective method for synchronization of cultured erythrocytic *Plasmodium falciparum*. *Parasitol Res*. 2008; 102:1195–1200. [PubMed: 18320226]
- Aley SB, Sherwood JA, Howard RJ. Knob-positive and knob-negative *Plasmodium falciparum* differ in expression of a strain-specific malarial antigen on the surface of infected erythrocytes. *J Exp Med*. 1984; 160:1585–1590. [PubMed: 6208311]
- Assaraf YG, Golenser J, Spira DT, Bachrach U. *Plasmodium falciparum*: synchronization of cultures with DL- α -difluoromethylornithine, an inhibitor of polyamine biosynthesis. *Exp Parasitol*. 1986; 61:229–235. [PubMed: 3082665]
- Bhakdi SC, Ottinger A, Somsri S, Sratongno P, Pannadaporn P, Chiuma P, Malasit P, Pattanapanyasat K, Neumann HP. Optimized high gradient magnetic separation for isolation of *Plasmodium*-infected red blood cells. *Malar J*. 2010; 9:38. [PubMed: 2012252]
- Collins WE. *Plasmodium knowlesi*: a malaria parasite of monkeys and humans. *Annu Rev Entomol*. 2012; 57:107–121. [PubMed: 22149265]
- Haynes JD, Moch JK. Automated synchronization of *Plasmodium falciparum* parasites by culture in a temperature-cycling incubator. *Methods Mol Med*. 2002; 72:489–497. [PubMed: 12125145]
- Heidrich HG, Mrema JEK, Vander Jagt DL, Reyes P, Rieckmann KH. Isolation of intracellular parasites (*Plasmodium falciparum*) from culture using free-flow electrophoresis: separation of the free parasites according to stages. *The Journal of parasitology*. 1982:443–450. [PubMed: 7047709]
- Hensmann M, Kwiatkowski D. Cellular basis of early cytokine response to *Plasmodium falciparum*. *Infect Immun*. 2001; 69:2364–2371. [PubMed: 11254594]
- Hoppe HC, Verschoor JA, Louw AI. *Plasmodium falciparum*: a comparison of synchronisation methods for in vitro cultures. *Exp Parasitol*. 1991; 72:464–467. [PubMed: 1902796]
- Hui G, Palmer KL, Siddiqui WA. Synchronization of *Plasmodium falciparum* in continuous in vitro culture: use of colchicine. *The American journal of tropical medicine and hygiene*. 1983; 32:1451. [PubMed: 6359912]
- Inselburg J. Gametocyte formation by the progeny of single *Plasmodium falciparum* schizonts. *J Parasitol*. 1983; 69:584–591. [PubMed: 6355424]
- Inselburg J, Banyal HS. *Plasmodium falciparum*: synchronization of asexual development with aphidicolin, a DNA synthesis inhibitor. *Exp Parasitol*. 1984; 57:48–54. [PubMed: 6420180]
- Jensen JB. Concentration from continuous culture of erythrocytes infected with trophozoites and schizonts of *Plasmodium falciparum*. *Am J Trop Med Hyg*. 1978; 27:1274–1276. [PubMed: 365006]
- Lambros C, Vanderberg JP. Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. *J Parasitol*. 1979; 65:418–420. [PubMed: 383936]
- Lelievre J, Berry A, Benoit-Vical F. An alternative method for *Plasmodium* culture synchronization. *Exp Parasitol*. 2005; 109:195–197. [PubMed: 15713452]
- Meng H, Zhang R, Yang H, Fan Q, Su X, Miao J, Cui L, Yang Z. In vitro sensitivity of *Plasmodium falciparum* clinical isolates from the China-Myanmar border area to quinine and association with polymorphism in the Na⁺/H⁺ exchanger. *Antimicrob Agents Chemother*. 2010; 54:4306–4313. [PubMed: 20643902]
- Mrema JE, Langreth SG, Jost RC, Rieckmann KH, Heidrich HG. *Plasmodium falciparum*: isolation and purification of spontaneously released merozoites by nylon membrane sieves. *Exp Parasitol*. 1982; 54:285–295. [PubMed: 6759149]
- Naughton J, Bell A. Studies on cell-cycle synchronization in the asexual erythrocytic stages of *Plasmodium falciparum*. *Parasitology*. 2007; 134:331. [PubMed: 17034650]

- Pasvol G, Wilson RJ, Smalley ME, Brown J. Separation of viable schizont-infected red cells of *Plasmodium falciparum* from human blood. *Ann Trop Med Parasitol*. 1978; 72:87–88. [PubMed: 350172]
- Paul F, Roath S, Melville D, Warhurst D, Osisanya J. Separation of malaria-infected erythrocytes from whole blood: use of a selective high-gradient magnetic separation technique. *Lancet*. 1981; 2:70. [PubMed: 6113443]
- Pfaller MA, Krogstad DJ, Parquette AR, Nguyen-Dinh P. *Plasmodium falciparum*: stage-specific lactate production in synchronized cultures. *Exp Parasitol*. 1982; 54:391–396. [PubMed: 6759150]
- Radfar A, Méndez D, Moneriz C, Linares M, Marín-García P, Puyet A, Diez A, Bautista JM. Synchronous culture of *Plasmodium falciparum* at high parasitemia levels. *Nature protocols*. 2009; 4:1899–1915.
- Ranford-Cartwright LC, Sinha A, Humphreys GS, Mwangi JM. New synchronization method for *Plasmodium falciparum*. *Malar J*. 2010; 9:170. [PubMed: 20565741]
- Scragg IG, Hensmann M, Bate CAW, Kwiatkowski D. Early cytokine induction by *Plasmodium falciparum* is not a classical endotoxin-like process. *European journal of immunology*. 1999; 29:2636–2644. [PubMed: 10458778]
- Smilkstein M, Sriwilaijaroen N, Kelly JX, Wilairat P, Riscoe M. Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. *Antimicrobial agents and chemotherapy*. 2004; 48:1803–1806. [PubMed: 15105138]
- Spadafora C, Gerena L, Kopydlowski KM. Comparison of the in vitro invasive capabilities of *Plasmodium falciparum* schizonts isolated by Percoll gradient or using magnetic based separation. *Malar J*. 2011; 10:96. [PubMed: 21501476]
- Trager W, Jensen JB. Human malaria parasites in continuous culture. *Science*. 1976; 193:673–675. [PubMed: 781840]

Highlights

- *P. falciparum* in vitro culture can be synchronized by storage at 4°C for 8–24 hours
- This simple refrigeration method can achieve >70% synchrony
- Synchronized parasites can be used for downstream work such as in vitro drug assay

Fig. 1. Growth dynamics of 3D7 parasites after synchronization at 4°C

The initial asynchronous parasite culture (at 0 h) with 43% rings, 37% trophozoites and 20% schizonts was stored at 4°C for 12 h and parasite growth was followed for two intraerythrocytic cycles. The graph shows relative proportion of parasites at different stages (quantified from a total of 500 parasites for each time point), while the micrographs are representative Giemsa-stained culture smears at corresponding time points.

Fig. 2. Growth curve of the same parasite culture aliquots synchronized by using 4°C refrigeration and sorbitol treatment

Synchronized ring-stage parasites from the two methods were seeded at 0.5% parasitemia in 24-well plates and parasite growth was monitored every 24 h. Each culture was replicated three times and the data represent means with standard deviations.

Fig. 3. Comparison of test drug dose-response curves between the two synchronization methods Representative dose-response curves of 3D7 and WB299 parasites to piperavaquine (PPQ) and dihydroartemisinin (DHA) (A and C) and to chloroquine (CQ) and quinine (QN) (B and D). Parasites were synchronized either by the 4°C refrigeration method (A and B) or the 5% sorbitol method (C and D). Data were fit to the sigmoid dose-response curves and are plotted as the means $\pm$ standard deviations of the means for three biological assays.

Table 1

Percentages (%) of ring-stage parasites (means ± standard deviations) in 3D7 culture after refrigeration at either 8°C or 4°C for different times.

Treatment temperature	Treatment time (h)			
	4	6	8	10
8°C	52.7±3.8	57.5±3.5	63.0±2.8	54.7±6.6
4°C	59.6±3.5	61.0±3.5	79.9±7.9	82.7±8.0
				80.4±10.8

Note: All parasites were cultured for 48 h after refrigeration treatments and percent of ring-stage parasites were determined from Giemsa-stained thin smears.

Table 2

Comparison of IC₅₀ values of parasites to four antimalarial drugs between the two synchronization methods (sorbitol treatment and 4°C refrigeration)

Strains	Drugs	IC ₅₀ values (nM)*		P value
		Sorbitol synchronization	4°C synchronization	
3D7	CQ	12.9 ± 4.3	18.3 ± 3.4	0.1632
	PPQ	4.9 ± 0.7	5.3 ± 0.3	0.4144
	DHA	0.7 ± 0.2	0.6 ± 0.1	0.4818
	QN	87.9 ± 10.8	95.5 ± 7.4	0.3716
HB3	CQ	6.8 ± 3.2	8.6 ± 2.3	0.4731
	PPQ	3.3 ± 1.0	5.1 ± 0.8	0.0716
	DHA	0.7 ± 0.3	1.2 ± 0.1	0.0520
	QN	273.0 ± 59.6	322.5 ± 77.0	0.3175
7G8	CQ	388.2 ± 71.0	421.6 ± 104.8	0.6714
	PPQ	25.1 ± 6.7	36.2 ± 5.5	0.0908
	DHA	0.8 ± 0.3	1.1 ± 0.2	0.2230
	QN	513.5 ± 57.3	600.3 ± 143.0	0.3844
Dd2	CQ	616.0 ± 121.3	521.4 ± 40.0	0.2327
	PPQ	34.5 ± 8.2	37.6 ± 8.5	0.6730
	DHA	1.1 ± 0.2	1.2 ± 0.3	0.6560
	QN	312.7 ± 19.9	277.7 ± 77.7	0.4918
WB299	CQ	193.5 ± 29.5	163.4 ± 15.4	0.1923
	PPQ	25.7 ± 3.9	20.9 ± 2.7	0.1545
	DHA	1.6 ± 0.1	1.8 ± 0.5	0.5342
	QN	386.0 ± 46.1	385.6 ± 19.3	0.9896
WB548	CQ	375.0 ± 81.8	323.1 ± 36.3	0.3720
	PPQ	15.8 ± 3.7	13.5 ± 2.2	0.4071
	DHA	1.4 ± 0.1	1.5 ± 0	0.1583
	QN	389.0 ± 71.1	292.9 ± 25.5	0.0923
WB183	CQ	326.6 ± 62.8	268.5 ± 45.8	0.2651
	PPQ	22.1 ± 1.1	18.7 ± 3.3	0.1657
	DHA	1.8 ± 0.5	1.7 ± 0.6	0.8354
	QN	571.7 ± 118.4	635.0 ± 83.0	0.4905
WB682	CQ	253.3 ± 61.7	317.0 ± 40.2	0.2084
	PPQ	21.4 ± 4.1	23.8 ± 4.4	0.5275
	DHA	2.8 ± 1.2	3.4 ± 0.9	0.5265
	QN	577.1 ± 122.8	468.0 ± 94.6	0.2898

* IC₅₀ values in nM are shown as mean ± standard deviation.