In Vitro Culture and Drug Sensitivity Assay of *Plasmodium falciparum* with Nonserum Substitute and Acute-Phase Sera

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The short-term in vitro growth of *Plasmodium falciparum* parasites in the asexual erythrocytic stage and the in vitro activities of eight standard antimalarial drugs were assessed and compared by using RPMI 1640 medium supplemented with 10% nonimmune human serum, 10% autologous or homologous acute-phase serum, or 0.5% Albumax I (lipid-enriched bovine serum albumin). In general, parasite growth was maximal with autologous (or homologous) serum, followed by Albumax I and nonimmune serum. The 50% inhibitory concentrations (IC$_{50}$) varied widely, depending on the serum or serum substitute. The comparison of IC$_{50}$s between assays with autologous and nonimmune sera showed that monodesethylamodiaquine, halofantrine, pyrimethamine, and cycloguanil had similar IC$_{50}$s. Although the IC$_{50}$s of chloroquine, monodesethylamodiaquine, and dihydroartemisinin were similar with Albumax I and autologous sera, the IC$_{50}$s of all test compounds obtained with Albumax I differed considerably from the corresponding values obtained with nonimmune serum. Our results suggest that Albumax I and autologous and homologous sera from symptomatic, malaria-infected patients may be useful alternative sources of serum for in vitro culture of *P. falciparum* isolates in the field. However, autologous sera and Albumax I do not seem to be suitable for the standardization of isotopic in vitro assays for all antimalarial drugs.

Cultivation of malaria parasites is an important tool for the understanding of parasite biology, biochemistry, molecular biology, immunology, and pharmacology. One of the applications of parasite cultivation is the in vitro drug sensitivity assay, which is a major tool for the screening of potential antimalarial drugs, the monitoring of drug sensitivity, and the detection of cross-resistance patterns against *Plasmodium falciparum* parasites (5, 25, 34). Although several assays have been developed, the in vitro cultivation technique of the erythrocytic stages of *P. falciparum* remains essentially the same as that originally described by Trager and Jensen (29). In this standard technique, the following components are required: *P. falciparum*-infected human erythrocytes, buffered RPMI 1640 medium, and human serum. The parasitized erythrocytes are incubated in a low-oxygen atmosphere at 37°C.

The culture medium is commercially available at a relatively low cost. The sources of infected blood abound in countries where malaria is endemic. In countries where malaria is not endemic, several reference clones and strains of *P. falciparum* are available in research laboratories. It has generally been accepted that nonimmune human serum is required for optimal parasite growth. However, the requirement for a regular supply of nonimmune human serum entails difficulties in conducting research in most of the African continent, where malaria transmission occurs at a high level throughout the year. Nonimmune human type AB-positive serum is relatively scarce and expensive in countries where malaria is not endemic. Furthermore, it is recommended that several units of serum from different donors be pooled to reduce batch-to-batch differences in the support of parasite growth (14). Additional problems include blood type compatibility and risks associated with the handling of infectious agents.

Because of these disadvantages, numerous alternative sources of sera and nonserum substitutes have been tested in the past (1, 6, 13, 15, 19, 27, 28, 37). Although some of these substitutes have been successfully used for the continuous cultivation of laboratory-adapted *P. falciparum* strains and clones, they are generally less effective than human serum and have not been adopted by many research laboratories. In a recent study, Ofulla et al. (21) have identified serum albumin and lipids as the key serum components that are necessary to sustain optimal parasite growth. Further studies have shown that a commercially available lipid-enriched bovine serum albumin, Albumax I (Gibco BRL), can replace human serum for the continuous in vitro cultivation of malaria parasites, leading to its routine use in several laboratories (2, 4, 7, 8, 31).

Another alternative source of serum is malaria parasite-infected patients themselves. It has been thought that semimmune human serum protects individuals who are continuously exposed to malaria parasites against malarial disease and therefore inhibits parasite growth (14). This assumption has not been proven experimentally through the quantitation and correlation of parasite growth inhibition, the level of acquired immunity, and the presence of antimalarial drugs in sera collected from indigenous populations. In addition, if this hypothesis were true, indigenous adults who have been exposed to the malaria parasite are expected to have acquired immune protection against further attacks of malaria. Contrary to this expectation, in many areas of endemicity in Africa, such as in Yaoundé, Cameroon, symptomatic malarial infection occurs frequently in both adults and children (24). It has also been shown that heat-inactivated serum from healthy, semi-immune African donors can support the growth of laboratory-adapted strains of parasites and fresh isolates and that acute-phase homologous serum may be useful for the continuous in vitro culture of reference strains (2, 20).
A preliminary in vitro study with lipid-enriched bovine serum albumin has reported that serum-free medium can be used instead of nonimmune serum to determine the level of drug activity (22). Autologous and homologous acute-phase sera from malaria parasite-infected patients and Albumax I have not been evaluated as alternatives for in vitro culture with fresh clinical isolates obtained from indigenous patients. In our present study, we (i) assessed the growth of clinical isolates of the malaria parasites in two different RPMI 1640 media during a single life cycle or two life cycles with nonimmune type A-Positive erythrocytes; Albumax I, autologous acute-phase serum, and homologous acute-phase serum with the aim of determining the best medium for short-term culture and in vitro drug assay and (ii) compared the in vitro activities of various anti-malarial compounds against fresh clinical isolates of *P. falciparum* using different sera or serum substitute with the aim of assessing whether these alternative sources can be used to standardize isotopic in vitro assays.

**MATERIALS AND METHODS**

**Clinical isolates.** Thirty fresh clinical isolates were obtained from symptomatic Cameroonian patients residing in Yaoundé before treatment. Eight were children between 5 and 14 years old; 22 were adults (≥15 years old; age range, 19 to 69 years). Our previous studies have shown that populations of patients in this age range in Yaoundé present with similar clinical and laboratory features (24, 26). The following inclusion criteria were set for this study: presence of signs and symptoms of acute uncomplicated malaria, monoinfection with *P. falciparum*, parasitemia of >0.2%, and no history of recent antimalarial drug intake confirmed by a negative Saker-Solomons urine test (18). Since this study is part of an ongoing clinical study designed to determine the clinical efficacy of first- and second-line drugs, pregnant women and patients with signs and symptoms of severe and complicated falciparum malaria, as defined by the World Health Organization (WHO) (33), were excluded. After informed consent was obtained, 10 ml of venous blood was collected in a tube coated with anticoagulant (EDTA) and in a tube not coated with anticoagulant. The patients were treated with either amodiaquine or sulfadoxine-pyrimethamine, which are first- and second-line drugs in Cameroon, respectively, and were monitored daily until they were cured. The study was approved by the Cameroonian National Ethics Committee.

**Drugs.** The following antimalarial drugs were obtained from the indicated sources: chloroquine sulfate, Rhone-Poulenc-Rorer, Antony, France; monodesethylamodiaquine, a biologically active metabolite of amodiaquine, Sapec S. A., Lugano, Switzerland; quinine hydrochloride, Sigma Chemical Co., St. Louis, Mo.; mefloquine hydrochloride, Hoffmann-La Roche, Basel, Switzerland; halo-fantrine hydrochloride, SmithKline Beecham, Hertfordshire, United Kingdom; dihydroartemisinin, a biologically active metabolite of arteisinin derivatives; Sapec S. A.; pyrimethamine base, Sigma Chemical Co.; and cycloguanil base, a biologically active metabolite of proguanil, Zeneca Pharma, La Defense, France; gentamicin (10 μg/ml), Sigma Chemical Co., St. Louis, Mo.; PABA- and folic acid-free RPMI 1640 medium. Two types of RPMI 1640 medium were used to cultivate the malaria parasites in two different RPMI 1640 media during a single life cycle or two life cycles with nonimmune type A-Positive erythrocytes; Albumax I, autologous acute-phase serum, and homologous acute-phase serum with the aim of determining the best medium for short-term culture and in vitro drug assay and (ii) compared the in vitro activities of various anti-malarial compounds against fresh clinical isolates of *P. falciparum* using different sera or serum substitute with the aim of assessing whether these alternative sources can be used to standardize isotopic in vitro assays.

**RESULTS**

Nine fresh clinical isolates were tested for in vitro growth in standard RPMI 1640 medium supplemented with nonimmune or autologous serum or Albumax I. All isolates adapted readily to the in vitro conditions, as shown by an adequate incorporation of tritium-labeled hypoxanthine (>3,000 cpm) during a 42-h incubation period (Fig. 1). The growth of most clinical isolates was optimal in the presence of autologous serum; the exceptions were two isolates (isolates 18/98 and 22/98) which grew better with Albumax I than with the autologous serum. One isolate (isolate 14/98) developed equally well with nonimmune serum and Albumax I. For parasite growth assays, the results either were expressed as counts per minute or were normalized to the growth of parasites in their corresponding autologous sera. The 50% inhibitory concentration (IC50) of the drug concentration corresponding to 50% of the uptake of [3H]hypoxanthine measured in drug-free control wells was determined by nonlinear regression analysis with Prism software (GraphPad Software, Inc., San Diego, Calif.). The IC50 determined for isolates cultivated in medium supplemented with different sera or serum substitute were expressed as the mean IC50 ratios. IC50 ratios were calculated from the following: Albumax I/nonimmune serum, Albumax I/autologous acid phase serum, and autologous acid phase serum/nonimmune serum. If the mean IC50 ratio between media supplemented with different sera or serum substitute was 100% or lower, the IC50 was considered to be equivalent. A comparison between nonimmune serum and Albumax I showed that three clinical isolates (isolates 13/98, 14/98, and 19/98) grew better (two- to eightfold better) with the nonimmune serum. For four other isolates, an opposite trend was observed, with higher (two- to sixfold) levels of hypoxanthine incorporation in the presence of Albumax I. Two isolates (isolates 6/98 and 16/98) grew almost equally well with nonimmune serum and Albumax I. Similar results were obtained with the isolates grown in PABA- and folic acid-free RPMI 1640 medium supplemented with nonimmune serum, autologous serum, or Albumax I over a 72-h incubation period.

The assessment of parasite growth in the standard RPMI
1640 medium supplemented with different sets of acute-phase sera showed that the parasites generally grew better with autologous or homologous acute-phase sera than with pooled nonimmune sera (data not shown). The homologous sera supported the growth of fresh isolates to a widely different extent, even surpassing the growth with autologous serum for some isolates. Parasite growth was not influenced by an ABO blood type incompatibility or storage of homologous sera at 4°C or −20°C.

The in vitro drug sensitivity patterns were determined for 11 clinical isolates with nonimmune serum, autologous acute-phase serum, and Albumax I. The differences in the IC$_{50}$ obtained with different sources of serum or serum substitute were relatively small for chloroquine and monodesethylamodiaquine (Table 1). Nonimmune serum gave the lowest IC$_{50}$ for chloroquine and monodesethylamodiaquine, generally followed by autologous serum and Albumax I. Wide variations in the IC$_{50}$s of the other test compounds were observed. The IC$_{50}$s of quinine and mefloquine differed considerably (nonimmune serum < Albumax I < autologous serum), with generally greater than a 2-fold difference between nonimmune serum and Albumax and greater than a 10-fold difference between nonimmune serum and autologous serum for quinine. The differences in the IC$_{50}$s of mefloquine were less pronounced. The order of the IC$_{50}$s of halofantrine was as follows: Albumax I < nonimmune serum < autologous serum. The IC$_{50}$s of dihydroartemisinin, pyrimethamine, and cycloguanil generally varied according to the following order: nonimmune serum ≤ autologous serum < Albumax I.

The IC$_{50}$ ratios are summarized in Table 2. According to our criterion of an equivalent IC$_{50}$, defined as an IC$_{50}$ ratio of between 0.50 and 1.50, the mean IC$_{50}$s obtained with Albumax I were not equivalent to any of the IC$_{50}$s obtained with nonimmune serum. However, equivalent IC$_{50}$s were obtained with Albumax I and autologous sera for chloroquine (IC$_{50}$ ratio, 1.10 ± 0.17) and monodesethylamodiaquine (IC$_{50}$ ratio, 1.41 ± 0.33). The IC$_{50}$s of dihydroartemisinin obtained with Albumax I and autologous sera were also similar, but the values were widely dispersed. The mean IC$_{50}$s of monodesethylamodiaquine were equivalent with nonimmune and autologous sera (IC$_{50}$ ratio, 1.10 ± 0.17). The IC$_{50}$s of halofantrine, pyrimethamine, and cycloguanil were also similar, but the IC$_{50}$ ratios were widely dispersed.

**DISCUSSION**

In previous studies, laboratory-adapted *P. falciparum* strains and fresh clinical isolates were successfully adapted and maintained in continuous culture with heat-inactivated semi-immune plasma or serum from African donors with no history of malarial infection in the preceding 3 weeks (2, 20). Our results further extend this observation and demonstrate that autologous and homologous acute-phase sera from malaria-infected patients also support the growth of fresh clinical isolates and often do so better than nonimmune sera. Thus, contrary to the unconfirmed assumption that local sera in areas of endemicity are unsuitable for parasite cultivation because they may contain antimalarial drugs and ill-defined immune factors (14), our results show that autologous and homologous sera from symptomatic patients may also be useful for parasite culture in the field. For most isolates, the in vitro growth attained maximal levels when the autologous acute-phase serum was added.
Clinical isolates may be readily adapted to short-term culture if the absence of antimalarial drugs is verified by a simple urine test (18). Our success rate in performing in vitro assays with nonimmune serum and fresh clinical isolates between 1993 and 1997 exceeds 92% in Yaoundé (23, 25). The in vitro studies conducted by Oduola et al. (20) and Binh et al. (2) with semi-immune and acute-phase sera to maintain cultures of laboratory-adapted strains and clones of *P. falciparum* support our observation that local sera may be useful for parasite cultivation. Our data suggest that both autologous and homologous acute-phase sera support parasite development for one or two life cycles and that the patients’ own acute-phase sera may be the best source of nutrients for the corresponding *P. falciparum* isolate, at least for short-term cultivation or for the initiation of culture of field isolates.

The use of Albumax I as a serum substitute for a long-term continuous culture has several advantages over the use of human serum. Albumax I costs less than human serum, is compatible with any blood type, and does not have a wide batch-to-batch difference, as is the case with serum. Growth of laboratory-adapted *P. falciparum* strains was similar in RPMI 1640 medium supplemented with hypoxanthine (0.2 mM) and nonimmune human serum or Albumax (4). Furthermore, Albumax has been used successfully to maintain a continuous culture of several *P. falciparum* strains in different laboratories (2, 4, 7, 8, 31). However, Albumax may not be suitable for in vitro drug assays since the IC50s of most antimalarial drugs tested in this study were consistently higher with the Albumax-supplemented medium than with the serum-supplemented medium. Using serum-free media containing 5 g of bovine albumin per liter and Cohn fraction V, Ofuilla et al. (22) also found that both chloroquine and quinine IC50s are, on average, 1.6 times higher than the values obtained with serum for 14 culture-adapted or fresh isolates. In their study, the ratio of amodiaquine IC50s (serum-free medium versus serum-supplemented medium) was 1.1 for 11 isolates.

### Table 1. Drug sensitivity patterns of *P. falciparum* isolates obtained with nonimmune or autologous sera or serum substitute (Albumax I)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Chloroquine</th>
<th>Amodiaquine</th>
<th>Quinine</th>
<th>Mefloquine</th>
<th>Halofantrine</th>
<th>Artemisinin</th>
<th>Pyrimethamine</th>
<th>Cycloguanil</th>
</tr>
</thead>
<tbody>
<tr>
<td>06/98</td>
<td>24.6</td>
<td>12.5</td>
<td>107</td>
<td>7.41</td>
<td>1.21</td>
<td>1.06</td>
<td>1.75</td>
<td>1.21</td>
</tr>
<tr>
<td>09/98</td>
<td>24.2</td>
<td>13.9</td>
<td>47.5</td>
<td>17.4</td>
<td>0.87</td>
<td>2.25</td>
<td>2.04</td>
<td>1.33</td>
</tr>
<tr>
<td>13/98</td>
<td>271</td>
<td>40.5</td>
<td>187</td>
<td>16.8</td>
<td>0.78</td>
<td>1.53</td>
<td>2.400</td>
<td>252</td>
</tr>
<tr>
<td>14/98</td>
<td>164</td>
<td>28.5</td>
<td>325</td>
<td>26.1</td>
<td>2.77</td>
<td>2.04</td>
<td>119</td>
<td>8.14</td>
</tr>
<tr>
<td>16/98</td>
<td>37.0</td>
<td>183</td>
<td>5.35</td>
<td>2.29</td>
<td>775</td>
<td>36.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18/98</td>
<td>26.2</td>
<td>13.4</td>
<td>24.8</td>
<td>14.9</td>
<td>4.00</td>
<td>1.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19/98</td>
<td>253</td>
<td>45.1</td>
<td>203</td>
<td>9.71</td>
<td>2.30</td>
<td>1.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20/98</td>
<td>120</td>
<td>16.5</td>
<td>233</td>
<td>4.68</td>
<td>0.95</td>
<td>0.93</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>21/98</td>
<td>30.5</td>
<td>277</td>
<td>4.36</td>
<td>2.86</td>
<td>70.6</td>
<td>25.8</td>
<td>414</td>
<td>22.1</td>
</tr>
<tr>
<td>22/98</td>
<td>158</td>
<td>19.7</td>
<td>247</td>
<td>4.28</td>
<td>2.9</td>
<td>0.25</td>
<td>2.46</td>
<td>123</td>
</tr>
<tr>
<td>24/98</td>
<td>382</td>
<td>22.6</td>
<td>11.6</td>
<td>16.7</td>
<td>3,710</td>
<td>333</td>
<td>49,800</td>
<td>1,880</td>
</tr>
</tbody>
</table>

* For each drug, the IC50s obtained for isolates with nonimmune human AB-positive serum (10% [vol/vol]), Albumax I (0.5% [wt/vol]), and autologous serum (10% [vol/vol]) are given in order (i.e., for each isolate, the top, middle, and bottom rows of data correspond to the IC50s obtained with nonimmune human AB-positive serum, Albumax I, and autologous serum, respectively). Amodiaquine and artemisinin refer to their biologically active human metabolites monodesethylamodiaquine and dihydroartemisinin, respectively.
supplemented media may be due to high levels of protein binding. In vivo, many antimalarial drugs are known to be highly bound to plasma proteins, notably, to albumin, which is the major component of plasma proteins and Albumax I. Quinine (70% to 95% protein binding), mefloquine (>98%), pyrimethamine (87%), and cycloguanil (75%), which had elevated IC₅₀, with Albumax-supplemented medium, are highly protein bound (35). Chloroquine is relatively less protein bound (50 to 70%), while monodesethylamodiaquine is highly protein bound (>90%). Protein binding alone does not explain the widely different IC₅₀ of some antimalarial drugs since the albumin concentration used in our experiments (for Albumax I, 0.5% [wt/vol], and for serum albumin, 10% [vol/vol]; the normal plasma albumin concentration is 35 to 50 g/liter) is similar in the two media.

Several possible factors may influence in the increase or decrease in the IC₅₀. First, as suggested by Ofuila et al. (22), the differential IC₅₀ may result, at least in part, from a higher affinity of antimalarial drugs for bovine albumin than for human albumin. Second, chloroquine and amodiaquine (highly hydrophilic, weak bases) undergo marked uptake into infected and uninfected erythrocytes (11, 30), which may explain the marked IC₅₀ obtained in our study. Third, albumin binds to lipophilic compounds, such as halofantrine, by means of hydrophobic binding forces, and plasma lipoproteins, notably, triglyceride, influence the IC₅₀ of halofantrine (12). Although the protein binding properties of halofantrine are still unknown due to its low solubility in water, these properties of albumin and lipids may explain the increased transport of halofantrine into infected erythrocytes in Albumax-supplemented medium and thus the lowering of the IC₅₀.

The standard in vitro test developed by WHO uses a mixture (9:1 [vol/vol]) of RPMI 1640 medium and the patient’s whole blood to determine the level of parasite growth in the presence of different drug concentrations (38). The results are interpreted by microscopic examination of thick blood smears. The results of the WHO in vitro test and those of in vitro assays that are based on the incorporation of tritium-labeled hypoxanthine are not comparable (36). The WHO test determines the maximal inhibitory concentration, while isotopic tests measure the IC₅₀. The former uses acute-phase plasma; the latter test is usually performed with nonimmune donor serum after washing of the infected erythrocytes. To our knowledge, no study has compared the two in vitro tests, but it is assumed that isotopic tests are more objective and accurate in determining the sensitivity levels (36). Although the standard WHO test uses whole blood, in our study autologous acute-phase sera did not yield consistent drug assay results compared with those obtained with nonimmune serum except for the results for monodesethylamodiaquine and, to a lesser extent, halofantrine, pyrimethamine, and cycloguanil. In most cases, the IC₅₀ obtained with autologous serum were increased more than two times compared with those obtained with nonimmune serum. An opposite trend would have been expected from the observation that the level of albumin in plasma is generally lower in malaria-infected patients than in healthy adults (9, 10), which should lead to a higher concentration of unbound drug available for schizontocidal action in the autologous serum. The most discordant result was observed with quinine; the IC₅₀ of quinine obtained with autologous serum were more than 10 times greater than the values obtained with nonimmune serum. The most likely explanation lies in the increased circulating concentration of the acute-phase plasma protein α-1 acid glycoprotein, which increases the level of protein binding of quinine in malaria parasite-infected patients (10, 16, 32). Furthermore, the level of protein binding of drugs is known to vary widely between patients (39). For these reasons, autologous sera are probably not useful for determination of the drug sensitivity pattern. Whether our observation extends to the WHO standard in vitro test that uses autologous plasma needs to be determined.

Our study demonstrates that autologous and homologous sera from patients with acute uncomplicated falciparum malaria are suitable for cultivation of field isolates and that their use results in a high growth rate compared with that obtained with nonimmune pooled sera and Albumax I during the first and second in vitro erythrocytic cycles. Our study further confirms the usefulness of Albumax I for the short-term cultivation of field isolates. Although autologous and homologous sera and Albumax I may serve as alternative sources of serum or serum substitute for the in vitro culture of fresh isolates in the field, at present, none of them seems to be suitable for in vitro drug sensitivity assays with the exception of autologous sera for monodesethylamodiaquine and Albumax I for halofantrine. However, if more data from a larger series of studies comparing Albumax I and nonimmune serum are accumulated, a reliable conversion factor in the form of IC₅₀ ratios can be calculated for antimalarial drugs for which the IC₅₀ ratios in this study were relatively low (chloroquine, monodesethylamodiaquine, mefloquine, and halofantrine). With such a conversion factor, threshold resistance values can be adjusted for Albumax I-supplemented medium. Other serum substitutes that provide adequate nutritional needs for the optimal growth.

### Table 2. Mean IC₅₀ ratios of antimalarial drugs obtained from nonimmune or autologous acute-phase sera or serum substitute (Albumax I) against fresh clinical isolates of *P. falciparum*

<table>
<thead>
<tr>
<th>Antimalarial drug</th>
<th>Alumex/nonimmune serum</th>
<th>Alumex/autologous serum</th>
<th>Autologous/nonimmune serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroquine</td>
<td>1.67 ± 0.46</td>
<td>1.10 ± 0.17</td>
<td>1.55 ± 0.48</td>
</tr>
<tr>
<td>Monodesethylamodiaquine</td>
<td>1.69 ± 0.58</td>
<td>1.41 ± 0.33</td>
<td>1.10 ± 0.17</td>
</tr>
<tr>
<td>Quinine</td>
<td>5.53 ± 6.01</td>
<td>0.44 ± 0.41</td>
<td>16.08 ± 11.28</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>1.65 ± 0.93</td>
<td>0.45 ± 0.24</td>
<td>3.55 ± 0.90</td>
</tr>
<tr>
<td>Halofantrine</td>
<td>0.41 ± 0.23</td>
<td>0.20 ± 0.12</td>
<td>1.26 ± 1.10</td>
</tr>
<tr>
<td>Dihydroartemisinine</td>
<td>4.65 ± 4.07</td>
<td>1.45 ± 3.38</td>
<td>3.38 ± 2.88</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>12.70 ± 16.14</td>
<td>6.91 ± 5.77</td>
<td>1.18 ± 0.74</td>
</tr>
<tr>
<td>Cycloguanil</td>
<td>7.62 ± 5.93</td>
<td>7.06 ± 6.16</td>
<td>1.25 ± 0.94</td>
</tr>
</tbody>
</table>

*IC₅₀ ratios are mean ± standard deviation (n = 6; n = 7 for pyrimethamine) ratios of IC₅₀ obtained with media supplemented with different sera or serum substitute (Albumax I).*

*The quinine IC₅₀ ratios are approximate since no growth inhibition was achieved in one assay with Albumax I and two assays with autologous sera. In these assays, the highest concentration in the assay (3,200 nmol/liter) was used to calculate the estimated mean ratio.*
of parasites and that do not bind strongly to antimalarial drugs may be necessary for the standardization of in vitro assays.

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