Assessment of the Drug Susceptibility of *Plasmodium falciparum* Clinical Isolates from Africa by Using a *Plasmodium* Lactate Dehydrogenase Immunodetection Assay and an Inhibitory Maximum Effect Model for Precise Measurement of the 50-Percent Inhibitory Concentration

Halima Kaddouri, Serge Nakache, Sandrine Houzé, France Mentré, and Jacques Le Bras

Centre National de Référence du Paludisme, Laboratoire de Parasitologie, AP-HP, Hôpital Bichat-Claude Bernard, and Université René Descartes, Paris, and INSEERM U738, Université Paris 7, and AP-HP, Hôpital Bichat-Claude Bernard, Département d’Épidémiologie, Biostatistique et Recherche Clinique, Paris, France

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The extension of drug resistance among malaria-causing *Plasmodium falciparum* parasites in Africa necessitates implementation of new combined therapeutic strategies. Drug susceptibility phenotyping requires precise measurement. Until recently, schizont maturation and isotopic in vitro assays were the only methods available, but their use was limited by technical constraints. This explains the revived interest in the development of replacement methods, such as the *Plasmodium* lactate dehydrogenase (pLDH) immunodetection assay. We evaluated a commercially controlled pLDH enzyme-linked immunosorbent assay (ELISA; the ELISA-Malaria antigen test; DiaMed AG, Cressier s/Morat, Switzerland) to assess drug susceptibility in a standard in vitro assay using fairly basic laboratory equipment to study the in vitro resistance of malaria parasites to major antimalarials. Five *Plasmodium falciparum* clones and 121 clinical African isolates collected during 2003 and 2004 were studied by the pLDH ELISA and the [8-3H]hypoxanthine isotopic assay as a reference with four antimalarials. Nonlinear regression with a maximum effect model was used to estimate the 50% inhibitory concentration (IC50) and its confidence intervals. The two methods were observed to have similar reproducibilities, but the pLDH ELISA demonstrated a higher sensitivity. The high correlation (r = 0.98) and the high phenotypic agreement (κ = 0.88) between the two methods allowed comparison by determination of the IC50. Recently collected *Plasmodium falciparum* African isolates were tested by pLDH ELISA and showed drug resistance or decreased susceptibilities of 62% to chloroquine and 11.5% to the active metabolite of amodiaquine. No decreased susceptibility to lumefantrine or the active metabolite of artemisinin was detected. The availability of this simple and highly sensitive pLDH immunodetection assay will provide an easier method for drug susceptibility testing of malaria parasites.

Resistance to currently available drugs is still a cause of therapeutic failures and of the persistence of a high level of morbidity due to malaria. Combined therapy with artemisinin derivatives plus amodiaquine or lumefantrine is now implemented in Africa. Epidemiological assessment of *Plasmodium falciparum* drug susceptibility is thus necessary to sustain health recommendations for malaria treatment and prophylaxis in countries where malaria is endemic. Even though molecular techniques are improving, few genes responsible for resistance have been identified. The in vitro assay, which allows the phenotypic determination of *P. falciparum* susceptibility to antimalarials, is thus an essential tool. The monoclonal structure of clinical *Plasmodium falciparum* isolates is the rule, and a mixture of susceptible and resistant parasites gives a biphasic concentration response (10, 22). As malaria parasites divide every 2 days, cloning is not a workable routine before testing for drug susceptibility. Our previous experience has shown that the in vitro response of a clinical isolate to chloroquine was monotonic at a frequency of >95%. This suggested that the majority of parasites which are present in the patient had homogeneous susceptibilities. The concentration-response relationship is a classical pharmacological sigmoid response, and the correct modeling of this response may give an accurate assessment of the drug susceptibilities of malaria parasites. Two methods are in common use for assessment of the in vitro parasite growth inhibited by drugs: schizont counting on thick films, known as the WHO Schizont Maturation assay (21), and incorporation of a radiolabeled nucleic acid precursor, [8-3H]hypoxanthine, known as the isotopic assay (6, 8). The high costs and/or the technical constraints of these methods have limited their use to a few centers. The sensitive quantification of major parasite proteins by sandwich enzyme-linked immunosorbent assay (ELISA) has recently gained particular interest (9, 13). The early interest in the use of *Plasmodium* lactate dehydrogenase (pLDH) production as a reflection of parasite growth came from the favorable characteristics of this enzyme, such as its structural differences from human LDHs (5) and the rapid decline of the levels of pLDH when the parasites die (15). pLDH, the last enzyme in the glycolytic pathway, is essential for energy production and parasite development (18). The first enzymatic drug susceptibility assays based on the measurement of the pLDH activity were encouraging, but they had poor sensitivities and the results were not interpretable if the initial parasite density was under 1% (3, 11). Further studies demonstrated a very high
sensitivity of the DELI assay, a double-site pLDH ELISA (4, 9, 12) for drug susceptibility determination. The DELI assay had a major limitation, in that the assay reagents are not available in a commercial kit. During the present study, we used an inhibitory sigmoid maximum effect (E_{max}) model to evaluate the performance of a commercially available pLDH ELISA, the ELISA-Malaria antigen test (DiaMed AG, Cressier s/Morat, Switzerland) with 5 P. falciparum culture-adapted strains and 121 clinical isolates. The isotopic assay was used as a reference.

MATERIALS AND METHODS

Parasites. (i) Reference culture-adapted strains. Five culture-adapted and cloned strains of P. falciparum were used: three chloroquine-resistant strains (strains W2 Indochina, FCM29 Cameroon, and 7GS Brazil) and two chloroquine-susceptible strains (strains 3D7 Africa and HB3 Honduras). All the clones except the Cameroon clone (established in our laboratory in 1979) were obtained from David Walliker, Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh, United Kingdom. The culture strains were cultivated until enough material was obtained and were synchronized with 5% D-sorbitol (Sigma) to obtain at least 90% ring forms before the in vitro assays could be run.

A first study was performed to compare the intra-assay variabilities of the two methods and to compare the results obtained by these two methods. This study measured the susceptibilities of the five cultivated strains to chloroquine and monodesethyl-amodiaquine (md-amod). The initial parasite density was 0.5%. Eight to 10 replicated tests with the same parasite suspension were run simultaneously for each strain, drug, and method of comparison (the ELISA and the isotopic assay). A second study estimated the sensitivities of the two methods by using strains 3D7 and 7GS and four antimalarial drugs (chloroquine, md-amod, lumefantrine and dihydro-artesomin (dh-artem)). The initial parasite density was 0.5%, 0.1%, 0.05%, 0.01%, and 0.005%.

(ii) Clinical isolates. A total of 121 clinical isolates collected during 2003 and 2004 from patients returning from Africa with P. falciparum infection were sent to us from French hospitals for in vitro susceptibility testing. Each isolate was tested for its susceptibility to one to four drugs, depending on the whole-blood volume available. The isotopic assay and the ELISA were run with the same parasite suspension for each isolate. As drug susceptibility testing is a routine method of analysis of the EDTA-anticoagulated venous blood sample taken for the diagnosis of malaria, no informed consent was required. These clinical isolates were used to compare the results obtained by the two methods and to evaluate the correlation between the results of the two methods. The sensitivities of the two methods were confirmed with one patient isolate. The initial parasite density was 0.5%, 0.1%, 0.05%, 0.01%, 0.005%, or 0.0025%.

In vitro assay. The following four antimalarial drugs in the appropriate solvent were distributed on 96-well tissue culture plates (4 by 24 wells) and dried: chloroquine disulfate (Sigma Aldrich), 12 to 3,200 nM; md-amod (Pfizer-Parke Davis), 7.5 to 1,920 nM; lumefantrine (Novartis), 1.25 to 320 nM; and dh-artem (Sanofi Aventis), 0.25 to 64 nM. Each batch of plates was controlled by measuring the susceptibilities of strains W2 Indochina and 3D7 Africa to the corresponding drugs, which were used within 1 year (chloroquine and md-amod), 4 months (lumefantrine), or 8 weeks (dh-artem), according to their previously determined shelf-lives (unpublished data). For each drug tested, three control wells were drug free, and each concentration was studied in duplicate or triplicate. Clinical isolates with at least a 0.2% parasite density were included in the study and were maintained at +4°C (for up to 48 h after collection) before a culture was started. The blood samples were washed three times with a solution of RPMI 1640 (Gibco, Invitrogen life Technologies) plus 25 mM HEPS (Sigma) and 25 mM NaHCO3 (Sigma). The blood samples were then resuspended in the same culture medium supplemented with 10% human serum (Abcys Biowest, Paris, France), but without hypoxanthine supplementation. If necessary, a dilution was performed by adding uninfected O-positive-group erythrocytes (EFS, Rungis, France) to obtain a 0.5% parasite density and a 1.5% hematocrit. The plates were incubated for 42 h at 37°C in a CO2 incubator (5% CO2 in air) and were then frozen and kept at −20°C.

Isotopic measurement. For the isotopic assay, 1 ml RPMI 1640 with [8-3H]hypoxanthine (40 mCi/liter; Amersham Biosciences) was added to 20 ml of the homogeneous parasite suspension (−0.4 μCi [8-3H]hypoxanthine/well) at the beginning of the in vitro assay. After the plates were thawed, the content of each well was harvested onto fiber filter disks (FilterMAT; Skatron Instruments). The fiber filter papers were washed and dried and mixed with 2 ml of scintillation fluid (OptiScint; Perkin-Elmer), and the level of parasite incorporation of radioactivity (in counts per minute) was measured with a liquid scintillation counter (Wallac 1410; Perkin-Elmer).

pLDH measurement by ELISA. For the ELISA, 1 ml RPMI 1640 without [8-3H]hypoxanthine was added to 20 ml of the homogeneous parasite suspension at the beginning of the in vitro assay. After the plates were thawed, the hemolyzed culture was homogenized by strong shaking of the plates for 15 to 20 min on a rotating platform. The success of the drug susceptibility assay and the appropriate volume of hemolyzed culture (from 1 to 20 μl) were previously determined for each clinical isolate by using a preliminary pLDH ELISA as a pretest. The pretest was performed with a couple of homogeneous culture wells: a 100% well (with no drug) and a 0% well (with the maximum concentration of chloroquine or dh-artem). Six wells for each isolate were needed on the ELISA plates: three dilutions (2 μl, 5 μl, and 15 μl) from both the 100% well and the 0% well were added to the wells of the ELISA plate, which contained lysis buffer, and two ELISA pLDH controls were used each time that an ELISA series was run. A test was designated as having failed if the ratio of the optical density (OD) for the 100% well to the OD for the 0% well was less than 1.7. For successful cases, the pLDH ELISA was performed as described below; the volume used was that which corresponded to the 100% well which had an OD closest to 1.5. The pLDH ELISA was done by using a commercial kit, as recommended by the manufacturer (ELISA-Malaria antigen test; DiaMed AG). Briefly, the ELISA plates were coated with a monoclonal antibody (MAB) against pan-Plasmodium LDH. Lysis buffer (100 μl) and, subsequently, 1 to 20 μl of hemolyzed culture or 50 μl of either a positive or a negative pLDH control were transferred to the ELISA plate wells. The ELISA plates were incubated for 1 h at 37°C with shaking and were then washed. A second incubation for 15 min at 37°C with 100 μl of streptavidinhorseradish peroxidase solution was followed by a last washing step. Enzyme activity was revealed by incubation for 15 min at 37°C with 100 μl of tetramethylbenzidine, the reaction was stopped with sulfuric acid, and the absorbance was read with a microplate spectrophotometer (LPO400; Bio-Rad) at 450 nm with a reference wavelength of 620 nm. The test was then scored as a failure if at least one of the following criteria was not met: (i) if the mean value for the 100% wells was <0.5 (OD) for the ELISA or <400 cpm for the isotopic assay or (ii) if the ratio of the mean value for the 100% wells to that for the 0% well was less than 1.7. These validity criteria correspond to a failure of culture. A third validity criterion linked to modeling is explained below.

Statistical analysis. The results of one test, for one drug and for one isolate or strain, are composed of 22 to 24 values of the counts per minute (isotopic assay) or the ODs (ELISA assay) for 10 different drug concentrations. For each drug concentration, the empirical mean of duplicate or triplicate values was analyzed. For each drug tested, a maximum of 10 mean values was obtained and is expressed as a percentage of the activity (hypoxanthine uptake or pLDH production) of the controls (mean of the values for the 100% wells). The resulting curve is used in nonlinear regression analysis which shows a decrease in the activity (in counts per minute) measured with a liquid scintillation counter (Wallac 1410; Perkin-Elmer).
enzymatic and isotopic assays, respectively, were compared by the nonparametric
Wilcoxon rank test. For all the clinical isolates for which interpretable results
were provided both by pLDH ELISA and by the isotopic assay, the means of the
IC50s of each drug tested were compared by the paired t test. Clinical isolates
were also identified as chloroquine resistant or chloroquine susceptible on the
basis of an IC50 cutoff of 100 nM. The IC50s between these two groups for each
of the three other drugs and for each assay method were compared by using a
Wilcoxon rank test. From these results, the prevalence of resistance or decreased
susceptibility to each drug was calculated and expressed with their nonparametric
confidence intervals. The relationship between the two measurement methods
was studied by use of the individual log IC50s of the four drugs tested by using the
Pearson correlation test and a Bland and Altman plot. The Bland and Altman
plot is used to study the level of agreement between methods and to show data
dispersion. In this plot, each point corresponds to one isolate tested with one
drug and represents the difference of the ELISA log IC50s (A) minus the isotopic
log IC50s (B) plotted against their mean, i.e., A/B versus (A+B)/2. This graph
displays, by horizontal lines, the mean difference of the log IC50s and the mean
difference plus and minus two times the standard deviation of the differences,
with the latter corresponding to the limits of agreement of the two techniques.
According to previous studies (2, 16, 17), a cutoff defining phenotypic resistance
is available only for chloroquine and is an IC50 of >100 nM by the isotopic assay.
Nevertheless, cutoffs for decreased susceptibility to md-amod, lumefantrine, and
dihydro-artem were estimated to be 80 nM, 150 nM, and 10 nM, respectively.

RESULTS

The intra-assay variabilities and the relative sensitivities of the isotopic assay and the pLDH ELISA for culture-adapted
strains are shown in Table 1 and Fig. 1, respectively. As shown
in Table 1, the IC50s obtained by the two methods were glo-
bally higher with the ELISA determination. The intra-assay
variabilities were rather similar for the isotopic assay (4.0 to
15.1%) and the enzymatic assay (4.4 to 13.8%). In this intra-
assay variability study, a total of 184 tests were run and ana-
lyzed by use of the Emax model. Among these 184 tests, mod-
ing of the drug effect failed in 12 cases (6.5%), but the results
for 7 of the 12 were accepted after it was considered that the
curve was very steep and therefore γ was fixed equal to 10.

![FIG. 1. Sensitivities of two modes of drug-response assessment. Tendency curves for the IC50s with upper confidence intervals determined by
the isotopic assay (open symbols, dotted lines) and the ELISA-Malaria antigen test assay (closed symbols, continuous lines) are shown for each
drug tested with two P. falciparum strains, strains 3D7 (A) and 7G8 (B). The IC50s of chloroquine (squares), monodesethyl-amodiaquine
(triangles), lumefantrine (diamonds), and dihydro-artemisinin (circles) were assessed at different initial parasite densities: 0.5%, 0.1%, 0.05%,
0.01%, and 0.005%.

TABLE 1. Comparison of replicate IC50 measurements performed by the isotopic and pLDH ELISA methods
with five reference clones of P. falciparum tested against two drugs

| Parasite strain | Drug       | No. of replicates | Isotopic assay | pLDH ELISA |
|-----------------|------------|------------------|----------------|------------|
|                 |            |                  | Geometric mean (range) | Geometric mean (range) | p<sup>b</sup> |
|                 |            |                  | IC50 (nmol/liter) | CV (%)     | IC50 (nmol/liter) | CV (%)     |
| 3D7 Africa      | Chloroquine| 10               | 33.5 (30.9–35.5) | 4.0         | 40.5 (34.5–47.4) | 10.3       | 0.005     |
|                 | md-amod    | 10               | 17.2 (15.5–18.9) | 5.9         | 18.5 (15.8–23.5) | 13.2       | 0.11      |
| HB3 Honduras    | Chloroquine| 10               | 52.4 (46.6–60.7) | 7.7         | 73.5 (62.9–85.0) | 12.1       | 0.018     |
|                 | md-amod    | 10               | 16.1 (14.4–18.0) | 6.9         | 20.4 (18.6–22.1) | 7.1        | 0.028     |
| W2 Indochina    | Chloroquine| 10               | 505 (341–609)   | 15.1        | 650 (534–745)   | 10.2       | 0.005     |
|                 | md-amod    | 10               | 39.5 (35.7–42.0) | 4.2         | 42.1 (36.2–49.5) | 10.5       | 0.28      |
| 7G8 Brazil      | Chloroquine| 10               | 516 (411–589)   | 9.8         | 706 (561–759)   | 9.1        | 0.008     |
|                 | md-amod    | 8                | 200 (191–221)   | 4.9         | 233 (211–263)   | 7.4        | 0.017     |
| FCM29 Cameroon  | Chloroquine| 8                | 820 (740–869)   | 4.9         | 1125 (822–1318) | 13.8       | 0.012     |
|                 | md-amod    | 8                | 210 (197–228)   | 5.8         | 246 (234–267)   | 4.4        | 0.018     |

<sup>a</sup> CV, coefficient of variation.
<sup>b</sup> P value of the Wilcoxon rank test for comparison of IC50s obtained by the two methods.
Further analysis assessed the sensitivity of the drug susceptibility assay. The results presented in Fig. 1 were determined for strains 3D7 and 7G8 at different initial parasite densities. The drug response was interpretable with initial parasite densities of 0.5% to 0.05% for the isotopic assay and 0.5% to 0.005% for the pLDH ELISA. Thus, the limits of sensitivity were attained at 0.005% for the pLDH ELISA (six of eight tests met the validity criteria) and at 0.05% for the isotopic assay (the eight tests met the validity criteria but were close to their limits). These results were confirmed by use of a clinical isolate (data not shown).

Of a total of 582 tests run with the clinical isolates, the modeling failed for 17 tests (2.9%), and the results of 8 tests were reconsidered and accepted by fixation of $\gamma$. Of all the tests that failed (92 of 582), failures were mainly determined before modeling, with the ratio of the OD for the 100% well to the OD for the 0% well in the pretest being less than 1.7. Among the 121 clinical isolates tested, 89 had an interpretable in vitro assay results both by the ELISA and by the isotopic method. Comparisons of the results for these isolates obtained by the two methods are illustrated in Table 2 and Fig. 2 to 4. Among the remaining isolates, in vitro analysis by the two methods failed for 18 of 121 isolates (14.9%). The response was interpretable only by the ELISA for 9 of 121 isolates (7.5%) and only by the isotopic assay for 5 of 121 isolates (4.1%). The failure rates of the isotopic assay and the enzymatic assay were similar, 22.3% (27 of 121 isolates) and 19% (23 of 121 isolates), respectively. The mean IC$_{50}$s were significantly higher for the ELISA than for the isotopic assay for chloroquine, md-amod, and lumefantrine ($P < 0.0003$) but not for dh-artem ($P = 0.061$) (Table 2). Examples of the dose-response curve fit by the $E_{max}$ model for a chloroquine-susceptible isolate and a chloroquine-resistant isolate analyzed by the two methods are shown in Fig. 5. The prevalence of resistance or decreased susceptibility determined for natural isolates by either the isotopic assay or the ELISA method was similar for chloroquine and also for md-amod (Table 2). For the African isolates...
studied by the pLDH ELISA, the prevalence of resistance was 0.62 (95% confidence interval, 0.5 to 0.73) for chloroquine (n = 79). The prevalences of decreased susceptibility were 0.11 (95% confidence interval, 0.05 to 0.22) for md-amod (n = 61), 0 (95% confidence interval, 0 to 0.08) for lumefantrine (n = 42), and 0 (95% confidence interval, 0 to 0.09) for dh-artem (n = 40). We compared the IC50s of the three other drugs for chloroquine-resistant and chloroquine-susceptible isolates tested by the two assay methods (Table 3). By both methods, md-amod was significantly less active against chloroquine-resistant isolates (P < 0.0001). Conversely, lumefantrine and dh-artem were significantly less active against chloroquine-susceptible isolates by the isotopic method of determination. However, the difference did not reach statistical significance by ELISA determination. The correlation between the log IC50s obtained by the two assays revealed a significant correlation for chloroquine (r = 0.94; n = 79; P < 0.0001), md-amod (r = 0.91; n = 61; P < 0.0001), lumefantrine (r = 0.78; n = 42; P < 0.0001), and dh-artem (r = 0.72; n = 40; P < 0.0001). For all tests with all drugs (n = 222), r was 0.98 (Fig. 3). We found a global kappa coefficient of 0.88 for all 222 measurements. In the Bland and Altman plot (Fig. 4), the mean difference in the log IC50s of the four drugs determined by the ELISA and the isotopic assay was 0.11 (limits of agreement, −0.22 to 0.44). This suggests that globally higher IC50s are obtained by the ELISA than by the isotopic assay. The limits of agreement that correspond to the confidence interval for the mean difference define the range containing 95% of the differences. No tendency for a greater or smaller difference between the two methods was shown as the IC50s increased.

**DISCUSSION**

Using a new statistical approach, we have shown in this paper that the commercially available pLDH ELISA (ELISA-Malaria antigen test) is suitable for the accurate in vitro measurement of drug susceptibility in *P. falciparum*. This pLDH ELISA assay is handy and easier to use than the previously available assays. Unlike the schizont maturation assay, it requires no microscopic skills. Unlike the isotopic assay, the success of the pLDH ELISA was obtained down to an initial parasitemia of 0.005% (approximately 250 parasites per microliter) with either cultivated strains or natural isolates. Two previously published sandwich ELISAs attained similar sensi-
tivities. The first one was the DELI assay (which also measures pLDH), which used two MAbs, one *P. falciparum* specific and the other pan specific for *Plasmodium* in a laborious preparation. The second one was the HRPII assay, which required more than one cycle of parasite maturation (72 h) to obtain enough parasite proteins for measurement. An advantage of high sensitivity is that it provides a better representation of the whole natural isolate populations, since samples with low levels of parasitemia may result from immune pressure or drug selection. It may also allow the easier detection of resistance in patients with low levels of recurrent parasitemia, e.g., in cases of therapeutic failure. In order to discard culture failures and to measure ODs over the correct range, we adjusted the volume of the culture submitted to immunocapture for each isolate according to the results of a pretest. The use of six wells per isolate for a preliminary pLDH ELISA was accurate and was adapted to series of tests. We developed a function for IC₅₀ estimation which has the main advantage that it associates a confidence interval with the IC₅₀ estimation. This function is based on nonlinear regression by use of an inhibitory *E*ₘₐₓ model. A second advantage is that the validity criteria for a test are operator independent and also that all measurements are kept in the estimation, which is therefore also operator independent. Nevertheless, some fittings are rejected on the basis of abnormal values or the presence of very few values between 100% and 0%, especially for a very steep decrease. For the latter case, the analysis may simply be preformed by use of a new estimation by fixing γ equal to its maximum value of 10.

Other fittings that present a biphasic response may require a more sophisticated analysis (22). For cases of imported malaria, the history of the cases is sometimes complex, as patients may have provided self-treatment or the transmission of isolates for culture may have been delayed, which may explain the majority of the culture failures that were observed. In the test comparisons, the intra-assay variabilities of both the pLDH ELISA and the isotopic assay with culture-adapted strains were rather similar. This validates the ability of the pLDH ELISA to provide precise IC₅₀ determinations. As for the clones, we found that for field isolates the geometric means of the IC₅₀s were higher by the pLDH ELISA than by the isotopic assay; the difference was significant for three of the four drugs tested. This observation is consistent with those from a previous study which also showed higher mean IC₅₀s of chloroquine and lumefantrine by the DELI pLDH ELISA than by isotopic determination (4). Unlike our results, the mean IC₅₀ of dh-artem determined by the DELI assay was lower than that determined by the isotopic assay. In this comparison study, the growth conditions of the parasites were strictly identical, and the concentration of 65 nM labeled hypoxanthine added in the isotopic assay was thought to be unlikely to enhance the metabolism of the parasites (1). Therefore, the higher values obtained by the ELISA could be explained only by the differences in metabolic pathways (hypoxanthine incorporation and protein production) used to assess the inhibition of growth (20). Despite the agreement that we found between the two methods (κ = 0.88), with the values being very close for chloroquine-susceptible isolates (Table 3; Fig. 5), the cutoffs for resistance and decreased susceptibility by the pLDH ELISA may have to be reconsidered. The mean ratio of the mean IC₅₀ determined by the ELISA and the mean IC₅₀ determined by the isotopic assay was 1.39 ± 0.59. The cost of the commercially available pLDH ELISA-Malaria antigen test must be considered. As underlined by Noedl et al. (14), the preparation and standardization of an ELISA require specific skills. The economy gained in running an in-house ELISA is then balanced with the constraints of controlling numerous steps and reagents. The costs of reagents for the pLDH ELISA are approximately the same as those for the isotopic assay, but new methods such as the SYBR green or PicoGreen fluorescence assays are much cheaper (7, 19). This is balanced with the cost of equipment, which is higher for the isotopic and the fluorescence assays than for the classical ELISA. Moreover, the applicability of the fluorescence assays to clinical isolates, where parasitized erythrocytes are often mixed with leukocytes, needs to be confirmed. The sensitivities of these fluorescence assays also need to be confirmed. The SYBR green assay proved successful for cultured strains with 0.2 to 2% initial levels of parasitemia (19).

According to our results, the prevalence of resistance to chloroquine is greater than 62% for all African isolates studied by the pLDH ELISA, and the rate of decreased sensitivity to the active metabolite of amodiaquine is less than 12%. This
decreased sensitivity to md-amod is only found within chloroquine-resistant isolates. As expected, according to the results of recent field studies, no decreased susceptibility to lumefantrine or to the active metabolite of artemisinin, which are the major components of the artemisinin-based combined therapies deployed in Africa, has been detected. These are preliminary data, and several field studies are in their early stages. As for rapid diagnostic tests, the availability of quantitative ELISA malaria antigen tests for surveillance for drug resistance may permit high-quality data to be obtained. Such data could be instrumental in assisting with the more rational use of antimalarial drugs.

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