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Shelf Life of Predosed Plates Containing Mefloquine, Artemisinin, Dihydroartemisinin, and Artesunate as Used for In Vitro *Plasmodium falciparum* Susceptibility Assessment▽

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ABSTRACT

The shelf lives of preserved antimalarial agent-predosed plates according to the type of wrapping and the temperature of storage were studied by measuring the 50% inhibitory concentrations of drug for *Plasmodium falciparum* 3D7. The shelf life of mefloquine was 8 weeks at 25°C; and those of artesunate, artemisinin, and dihydroartemisinin were a minimum of 24, 12, and 8 weeks, respectively, at 4°C.

Other Sections▼

Plasmodium falciparum antimalarial drug resistance contributes to morbidity and requires accurate surveillance. The results of in vitro drug susceptibility testing of *P. falciparum* have been reported to be disparate (2, 5). Drug susceptibility was mainly assessed by determination of the in vitro drug inhibition of parasite growth (8). Ready-to-use predosed plates are produced under WHO control (17), but the conservation of antimalarial activity over time is a concern.

The present study explored the influences of the storage temperature and the type of wrapping of preserved predosed plates on the 50% inhibitory concentrations (IC_{50}s), which were compared to the IC_{50}s obtained for predosed plates dried for 24 h as a reference, and determined the shelf life of each antimalarial drug.

Mefloquine hydrochloride was obtained from Hoffmann-La Roche (Basel, Switzerland), artesunate was from Aventis (Gentilly, France), and artemisinin and dihydroartemisinin (DHA) were from Sigma-Aldrich Company (St. Louis, MO). The final dilutions of the drugs in water (artemisinins) or methanol (mefloquine) from methanol stock solutions (which were identical throughout the study) were distributed with a MicroLab device (Hamilton, Reno, NV) in 2.5- to 25 μl volumes into 96-well culture plates and dried. The final concentrations ranged from 2.5 to 400 nM for mefloquine and from 0.25 to 40 nM for artemisinin and its derivatives (artemisinins).

One predosed plate dried for 24 h was tested, and the time for that plate was considered week 0. To test the storage conditions, the preserved predosed plates were divided: they were unwrapped, wrapped in aluminum foil and then in a plastic bag closed with a piece of self-adhesive tape (standard WHO packaging), or sealed in an aluminum bag with a desiccant. Each group was divided into three parts; and each part was stored at 4°C (except for the plates in an aluminum bag), 25°C, and 37°C, respectively.

Plasmodium falciparum strain 3D7 was cryopreserved and thawed before each measurement. It was cultivated in erythrocytes resuspended in RPMI 1640 medium supplemented with 10% human serum (Biowest, Nuaillé, France) at 37°C in a 5% CO_2- 5% O_2-85% N_2 atmosphere until a minimum parasite density of 1% was achieved (16). The susceptibilities to the antimalarial drugs were determined after suspension in the same medium and addition of uninfected erythrocytes to obtain 0.3% parasitemia and 1.5% hematocrit. The culture obtained within three asexual cycles after thawing contained a minimum of 90% ring forms and did not require synchronization (14).

The isotopic microtest described previously (12) was used for the in vitro assay. IC_{50}s were determined by nonlinear regression analysis with an inhibitory sigmoid maximum-effect model. The estimated parameters were the IC_{50} and its confidence interval (CI) (12).

Each condition except for the plates in an aluminum bag was tested at 1, 2, 3, 4, 6, 8, 12, 16, and 24 weeks; the plates in an aluminum bag were tested every two attempts (i.e., at 1, 3, 6, 12, and 24 weeks). As reference, a plate on which drugs had been distributed 24 h earlier and dried was tested concomitantly.

The repeatability analysis relied on a type III analysis of variance for investigation of the parasite suspension, drug, and experimental factors. We express the results as the coefficients of variation (CVs). CVs defined the limits of the plates' acceptabilities. We then investigated the evolution of the IC_{50} over time compared to these limits.

The repeatability was assessed by the use of 16 independent IC_{50} measurements. The overall variation was between 14% (mefloquine) and 25% (artemisinin and derivatives), which were further considered the limits of acceptance. The plate was then considered not valid if the IC_{50} ± 95% CI was off from the limits of acceptance (IC_{50} ± CV for the reference plate) for two successive measurements for a given drug and the condition was not tested again.

At 37°C, whatever the conditions of packaging or the drug used, the plates were no longer valid after 1 week. The validity was longer at 4°C than at 25°C ($P = 0.002$) for the artemisinins (Fig. 1) but not for mefloquine (Table 1). The packaging had no influence on the shelf life (Table 1). The results for the predosed plates altered with time ($P < 0.0001$) more rapidly for the artemisinins ($P < 0.0001$) than for mefloquine ($P = 0.04$). In addition, the effect of time on the degradation of the plates was higher at 25°C ($P < 0.0001$) than at 4°C ($P = 0.04$) (Fig. 1). The limits of the shelf life at 4°C were 6 to 8 weeks for mefloquine, 8 weeks for DHA, 12 weeks for artemisinin, and 24 weeks for artesunate (Table 1). In the case of storage at 25°C, the limits were 6 to 8 weeks for mefloquine, 4 weeks for DHA, 8 weeks for artemisinin, and 12 weeks for artesunate, according to the packaging (Table 1).

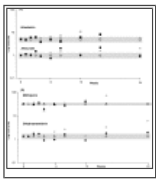

FIG. 1.
Variations in IC_{50}s of four antimalarials for *P. falciparum* clone 3D7 over time according to two shelf conditions until the limit of the shelf life: artemisinin and artesunate (A) and mefloquine and dihydroartemisinin (B). To make the IC_{50} evolution comparable (more ...)

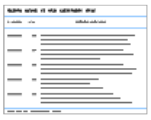

TABLE 1.
Shelf lives of predosed plates according to drug, storage temperature, and packaging

One study on the conservation of predosed plates has been published (11). The exploration of drugs by measurement by high-pressure liquid chromatography is not possible due to the level of detection by that method. The only possible control is a biological assay. We observed that the shelf life is often better at 4°C without packaging.

The IC_{50} obtained by a microtest is the result of many interactions (3, 10). In fact, if practical procedures that have been suggested previously (18) are used, numerous variations in the details of these protocols make it impossible to compare these IC_{50} values between laboratories worldwide.

We consider the IC_{50} of a fresh dried plate (24 h) to be reference of the plate's stability. We had to define the limits of acceptability in the variation of the IC_{50}s in different experiments. In previous studies, the CVs were low (less than 10%) (1) or high (10 to 40%), according to the test, the strain, and the drug used (7). CVs that depended on the drugs used (9) were chosen as limits of acceptability for the molecule.

The packaging in an aluminum bag with desiccant, which could allow degradation as a result of exposure to light and humidity to be avoided, was not better than the packaging recommended by WHO. The exact cause of the quick degradation (in terms of a decrease in bioavailability or chemical degradation) observed at 37°C and the slower degradation at 25°C has not been determined (13). The quality of the plates supplied or their incorrect use could be responsible for the false drug susceptibility or resistance results (4). Even with *Plasmodium* lactate dehydrogenase or histidine-rich protein 2 measurements (6, 14), the culture of parasites with drugs still remains a factor limiting the collection of sound data. The first step for the retrieval of reproducible and reliable results would be the availability of a ready-to-use method (15). As very few studies on the validation of plates for the study of antimalarial drug susceptibility are available, no comparison may help to clearly identify the weakness of using either predosed plates or plates prepared at the time of use.

In conclusion, we recommend drugs with very different shelf lives not be included in the same plate and that the drug with shortest shelf live be the plate life. When a drug with a very short shelf life is to be tested, it is better to prepare the plates at the time of use.

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FOOTNOTES

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