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Colorimetric and thin-layer chromatographic methods for field assay of chloroquine and its metabolites in urine*

D. L. MOUNT,[1] L. C. PATCHEN,[1] S. B. WILLIAMS,[2] & F. C. CHURCHILL[3]

Three field-adapted methods for the quantification of the antimalarial drug chloroquine are described. Two of the methods are modifications of the Haskins test and are based on ion-pair formation between chloroquine and methyl orange in either dichloromethane or chloroform. Absorbance values measured at 420 nm with a hand-held, battery-operated filter photometer were linearly related to chloroquine concentrations in urine up to 100 μmol/l (32 μg/ml) for both methods. The contribution of the desethylchloroquine metabolite to the measured absorbance for both methods is less than that of chloroquine; the relative sensitivity for this metabolite is about 50% of that of chloroquine for both methods. The detection limit for modification I is 1 μmol/l (0.3 μg/ml), while that for modification II is 3 μmol/l (1 μg/ml). A single dose of chloroquine diphosphate (300 mg as base) administered to each of three volunteers yielded detectable levels by modification I of chloroquine in the urine for 28 days after dosing. Results for the colorimetric methods correlated well with the liquid chromatographic reference method used. The related thin-layer chromatographic method confirmed the presence of chloroquine and desethylchloroquine in the urine and permitted independent estimation of the concentration of these two compounds if desired. The two colorimetric methods may be used in remote locations where no electricity is available.

Measuring the levels of antimalarial drugs and metabolites in blood and urine is important in malaria field studies for such purposes as monitoring chemoprophylaxis compliance and, in concert with *in-vitro*, drug-sensitivity testing, for characterizing the incidence of drug resistance in strains of *Plasmodium falciparum*. Methods using sophisticated analytical instrumentation may be interfaced with field studies by approaches such as preservation of blood samples on filter-paper to permit transport of samples to the laboratory in an envelope by regular mail (*1*, *2*). It is often necessary or desirable, however, to obtain analytical results without delay in the field.

Colorimetric methods for field assay of chloroquine (Cq) in the urine have been available for many years and include the Dill–Glazko test (*3*), the Haskins test (*4*), and the Wilson–Edeson test (*5*). These tests are not specific and are less sensitive than is desirable. Of these three tests, the Dill–Glazko has probably been most used, because of its simplicity; however, several research groups have concluded that this test is not sufficiently sensitive or reliable to be useful in assessing history of chloroquine use in the field (*6*, *7*). Rombo et al. evaluated these tests and concluded that the Dill–Glazko test should not be used because of its insensitivity and unreliability (*8*). Bergqvist et al. (*9*) introduced a simple photometric method that utilizes bromthymol blue as the ion-pair extracting agent for assaying chloroquine in urine. This method

* From the Control Technology Branch, Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA 30333, USA.

[1] Research Chemist, Control Technology Branch.

[2] Biological Technician, Control Technology Branch.

[3] Chief, Control Technology Branch. Requests for reprints should be addressed to this author.

is linear for concentrations beyond 300 μmol/l and has a detection limit of 10 μmol/l; it is somewhat more selective than the older methods but no more sensitive than the Wilson–Edeson or Haskins methods (*4, 5, 8*). Thin-layer chromatographic (TLC) methods have been applied to provide sensitive and selective quantification of Cq and its major metabolite, desethylchloroquine (DECq), in the laboratory, although compromises are necessary to adapt such TLC methods for field use (*10*).

We set out to develop a family of field methods for urine analysis that would permit the sensitive detection of Cq and metabolites and, if desired, confirm the presence of Cq and DECq. We report two methods that are modifications of the Haskins test, one optimized for sensitivity (designated Haskins MMI), the other for simplicity (designated Haskins MMII); both are field-adapted and use a hand-held, battery-operated, filter photometer for quantification. Further, we report a related and complementary, field-adapted, TLC method that can be used to confirm the presence of Cq and DECq and/or to screen for the presence of other antimalarial drugs in urine.

MATERIALS AND METHODS[a]

A Spectronic 20 spectrophotometer[b] was used to make initial colorimetric measurements and to verify the photometric accuracy of the battery-operated, hand-held, filter photometer[c] with a 420-nm filter for colorimetric quantification. The TLC plates were KGF silica-gel plates[d] and the ultraviolet lamp used to visualize the compounds on the fluorescent background was the UVS-11 mineral-light model.[e] Specific gravities of urine were determined using a urinometer (item number 1525).[f]

Reagents and standards

Chloroquine and desethylchloroquine standards were available from earlier studies and had been kindly donated,[g] as were the SAG-470 and SAG-10 defoamers which are commercially available.[h] Methyl *tert*-butyl ether,[i] methanol,[i] chloroform,[i] methylene chloride,[i] dimethyl sulfoxide,[j] methyl orange,[j] and 2,6-dichloroquinone-4-chlorimide[j] were obtained commercially. All the other chemicals were of reagent grade or better and available commercially. The pH 8 phosphate buffer contained 324 g of $K_2HPO_4.3H_2O$ and 10 g of KH_2PO_4 in 1 litre of water.

Procedure for modification I of the Haskins test (Haskins MMI)

Pre-field preparations. The 15-ml screw-cap centrifuge tubes were charged with about a 1-ml volume of dry trisodium phosphate powder and 1.3 ml of methyl *tert*-butyl ether (MTBE). Screw-cap culture tubes (100 mm × 13 mm O.D.) were charged with 4 ml of methylene chloride and 2 ml of nominal 0.1% aqueous methyl orange solution containing 5% boric acid. The methyl orange solution had been allowed to stand for at least 3 hours and then filtered before use.

Field assay. The 5-ml quantities of urine from each subject were transferred into a precharged 15-ml centrifuge tube. The tubes were capped and shaken by hand for 1 min. Many tubes may be shaken simultaneously. The layers were allowed to separate for at least 30 sec, and 1 drop of aqueous defoamer (SAG-470 or SAG-10) was added to each tube. The tube was agitated gently and set aside for 10–15 min to allow the MTBE phase to clarify. (The use of 20 inversions of each sample tube, as described below for the Haskins MMII procedure, was shown later to provide equivalent extraction and result in more rapid separation of layers.) A centrifugation step may be used rather than the defoamer if facilities permit. As much as possible of each clarified MTBE layer was transferred by Pasteur pipette into a corresponding tube precharged with methyl orange solution and dichloromethane. (If greater precision is desired, a specific aliquot may be transferred.) These tubes were capped and shaken for 30 sec. After the two phases separated, each tube was placed in turn in the battery-operated, filter photometer and an absorbance (or percentage transmittance) reading taken. If the dichloromethane layer appeared turbid, the tube was held in the palm of the hand until the layer cleared. Each tube was rotated in the filter photometer and the reading taken at minimum absorbance to minimize the effect of irregularities in the tubes and beading of the aqueous methyl orange of the sides of the tube at the level of the dichloromethane layer. Each value was recorded and compared with a prepared standard

[a] Use of trade names and commercial sources is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

[b] Bausch & Lomb, Rochester, NY, USA.

[c] Chemetrics Inc, Calverton, VA, USA.

[d] Whatman Co., Clifton, NJ, USA.

[e] Ultra-Violet Products Inc., San Gabriel, CA, USA.

[f] Becton, Dickinson & Co., Parsippany, NJ, USA.

[g] Sterling-Winthrop, Rensselaer, NY, USA.

[h] Union Carbide Company, Danbury, CT, USA.

[i] Burdick & Jackson Laboratories, Muskegon, MI, USA.

[j] Aldrich Chemical Company, Milwaukee, WI, USA.

curve to convert the filter photometer reading to a concentration value. The procedure was performed in a well ventilated area to minimize exposure to dichloromethane vapours. Chloroform may be substituted for dichloromethane in the procedure.

Procedure for modification II of the Haskins test (Haskins MMII)

Pre-field preparations. Screw-cap culture tubes (100 mm×13 mm O.D.) were charged with 1 ml of pH 8 phosphate buffer and 2-ml of chloroform.

Field assay. Each 2-ml quantity of sample urine was added to a precharged culture tube, and the capped tube was inverted 20 times. The phases were allowed to separate (1–2 min), and the aqueous phase was aspirated by Pasteur pipette. A 1-ml quantity of nominal 0.2% methyl orange in 5% boric acid solution was added and the tubes capped and inverted 20 times. (The solution had been filtered similarly to the 0.1% solution.) The phases were allowed to separate and then a glass-wool swab used to clarify the chloroform layer. The absorbance (or percentage transmittance) of the chloroform layer was then read using the battery-operated, hand-held, filter photometer equipped with a 420-nm filter. Each value was recorded and compared with a prepared standard curve to convert the filter photometer readings to a concentration value. As with the Haskins MMI procedure, exposure to the chlorohydrocarbon solvent should be minimized by using good ventilation.

Procedure for thin-layer chromatographic assay

A 5-ml quantity of urine was transferred to a 15-ml screw-cap centrifuge tube charged with about 1 ml of trisodium phosphate powder and 1 ml of MTBE. Partitioning into the MTBE layer was effected as for Haskins MMI. Either the defoamer or centrifuging may be used to separate the layers. As much as possible of the clarified MTBE layer was drawn into a disposable Pasteur pipette and spotted carefully onto a TLC plate heated to about 70 °C on a hot-plate at a rate sufficiently slow to ensure that the final diameter of the spot was 1 cm or less. Plates up to 20 cm×20 cm may be used as long as they fit entirely on the hot-plate. When application of samples and standards was complete, the plate was placed into a developing tank containing a mobile phase that contained 98 parts methanol and 2 parts of 15 mol/l ammonia. The plate was developed to a height of about 15 cm over a period of 60 min, removed from the tank, and air-dried. The plate was then sprayed with a solution of 0.1% 2,6-dichloroquinone-4-chloroimide in 10 parts of sodium bicarbonate saturated dimethyl sulfoxide in 90 parts of chloroform. The solution should be stored in a cool, dark place when not in use. The plate was air-dried and then placed on the hot-plate at 70 °C to develop. The size and intensity of the spots were compared with standards to estimate the amount of Cq and DECq, using either the grey to greenish-blue spots on a white background under ordinary light or dark spots on a fluorescent-blue background under a short-wave (254 nm), hand-held, ultraviolet lamp. The latter visualization method may be used even if the plate is not sprayed with the spray reagent.

Procedure for liquid chromatographic assay

The liquid chromatographic method has been previously described (*1*).

Volunteer dosing and sampling regimen

The three volunteers were dosed and samples taken in two phases. In Phase 1 each volunteer was given 1 tablet of chloroquine disphosphate (300 mg as base), and urine and blood samples were collected at intervals over a 4-week period. Phase 2 began at the end of the fourth week of Phase 1. Four weekly doses (1 tablet per dose) were given. After the fourth dose, urine and blood samples were collected on a similar schedule to that for Phase 1, except that additional blood samples were drawn. Specific gravities were measured for urine samples collected in the second phase of the experiment.

RESULTS

Characterization of Haskins MMI

The calibration curve for the Haskins MMI method is linear over the range 0 to 100 μmol/l. For concentrations above 30 μmol/l (absorbance of about 1), either a dilution of the methylene chloride layer is made or a volume of urine smaller than 5 ml is taken for a subsequent analysis. The detection limit for the method is 1 μmol/l. Although the practice of taking as much of the MTBE layer as possible is somewhat less precise than taking a specific aliquot, this approach is more convenient and gives adequate precision in our hands as seen by comparison with the high-performance liquid chromatographic (HPLC) reference method (see below).

Characterization of Haskins MMII

The calibration curve for the Haskins MMII method is linear over the range of 0 to 100 μmol/l.

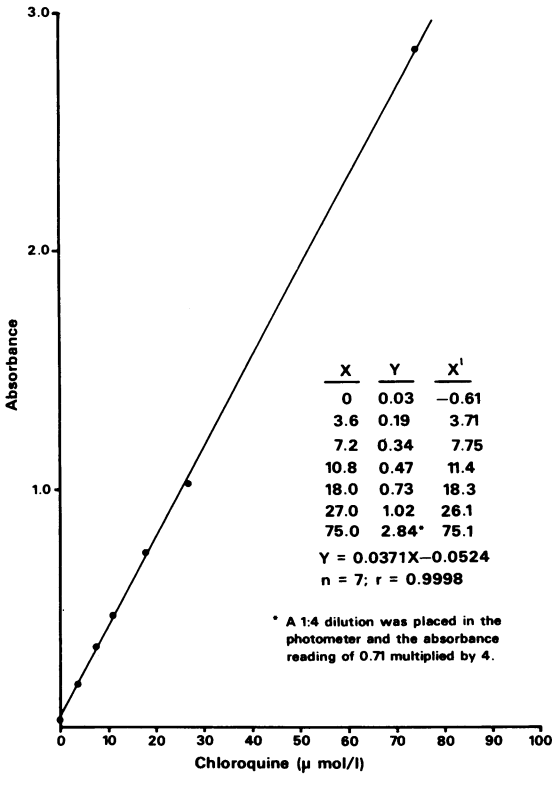

Fig. 1. Calibration curve for the Haskins MMII method.

Samples found to contain above 30 μmol/l are treated as indicated above for the Haskins MMI method. The detection limit for Haskins MMII is 3 μmol/l, which corresponds to an absorbance value 4 standard deviations above the mean blank value for the urines of a population of 36 individuals. Fig. 1 illustrates a standard curve for Haskins MMII. Experience has indicated that a given methyl-orange solution may be used for at least one week before recalibration becomes necessary.

Characterization of the TLC method

The TLC method uses the same initial extraction procedure as does Haskins MMI. The method separates Cq and DECq well, yielding an R_f of 0.36 for the former and 0.21 for the metabolite. The method is sensitive to a level of about 3 μmol/l (1 μg/ml) and allows estimates of the two analytes by comparing the spot size and intensity for samples and standards. TLC allows confirmation of the presence of Cq and DECq because of its greater specificity when compared with the modified Haskins tests. The presence of spots at R_f 0.36 and 0.21 is strong confirmation of a history of Cq usage. Desethylamodiaquine and quinine have R_f 0.41 and 0.53, respectively, in this TLC system.

Volunteer studies

The data from the volunteer studies are summarized in Tables 1 and 2. Table 1 contains data from the study in which each volunteer received only one dose of Cq diphosphate (300 mg as base). Urine and blood samples were drawn at the indicated intervals and analysed by Haskins MMI (urine), TLC (urine), and HPLC (blood and urine). Table 2 contains data in the allied study in which the same volunteers were dosed 4 times, at weekly intervals, with Cq diphosphate (300 mg as base); urine and blood samples were drawn as indicated for 4 weeks subsequent to the fourth and final dose. In this study urine samples were analysed by TLC, both modifications of the Haskins test, and HPLC; blood levels of Cq and DECq were assayed by HPLC.

Values in Table 1 from the battery-operated, filter photometer were compared with those from the laboratory spectrophotometer ($y=1.009x+0.1981$; $n=19$, $r=0.9807$) when these two instruments were used for quantification in analysis by Haskins MMI. The values from Haskins MMI compared well with the HPLC results for Cq ($y=0.9640x+0.4024$; $n=18$, $r=0.9959$). The corresponding comparison with HPLC results summing Cq and DECq gave a linear relationship characterized by the equation $y=0.8251x+0.2366$; $n=18$. The correlation coefficient was 0.9938.

The contribution of the metabolite DECq to Haskins MMI results was found to be 0.50 of that for Cq as determined by comparison of absorbances for equivalent molar concentrations of Cq and DECq when analysed by the Haskins MMI method. On this basis the Haskins MMI result was correlated with a value arrived at from HPLC data by adding half of the DECq value to the Cq value. For this comparison $y=0.8903x+0.3039$ with $n=18$ and a correlation coefficient of 0.9953.

A comparison of Haskins MMI values with HPLC Cq values for urine was also made for the data of Table 2 ($y=1.121x+0.144$; $n=21$, $r=0.9922$). The corresponding values for comparison of Haskins MMI with the summation of HPLC values for Cq and DECq were $y=0.853x+0.285$; $n=21$, $r=0.9867$. Inclusion of the sensitivity factor of 0.50 for DECq gives the least-square relationship $y=0.9705x+0.1899$ with $n=21$ and $r=0.9900$. Thus, for these data the Haskins MMI test provides a molar con-

Table 1. Results of urine and blood tests on three volunteers after one dose of chloroquine diphosphate

Volunteer and sampling time (days)	Urine results (μmol/l)					Blood results (nmol/l)		
	Haskins MMI[a]		HPLC			HPLC		
	Spectro-photometry	Filter photometry[b]	Cq	DECq	Cq + DECq	Cq	DECq	Cq + DECq
SW: 0	<1.0	<1.0	<0.3	<0.3	<0.6	<15	<15	<30
3	38.8	40.7	41.9	6.90	48.8	272	91.9	364
7	6.35	7.26	6.17	1.00	7.17	118	70.2	188
10	5.88	5.79	6.20	1.41	7.61			
14	6.42	7.48	6.07	1.31	7.38			
21	3.38	3.91	3.44	1.10	4.54			
28	1.00	1.06	1.35	0.58	1.93			
CD: 0	10.7[c]	9.17[c]	<0.3	<0.3	<0.6	<15	<15	<30
3	21.7	17.4	18.2	5.26	23.5	216	85.3	301
7	11.3	11.8	10.4	2.13	12.5	76.1	67.4	144
10	8.42	8.70	8.17	1.89	10.1			
14	4.04	5.32	4.26	1.34	5.60			
21	3.00	2.91	2.91	0.89	3.80			
28	1.13	1.16	1.63	0.89	2.52			
FC: 0	<1.0	<1.0	<0.3	<0.3	<0.6	<15	<15	<30
3	18.0	12.8	14.2	1.27	15.5	225	30.6	255
7	12.6	11.4	10.7	0.65	11.4	148	24.4	173
10	8.14	7.48	7.61	1.10	8.71			
14	5.95	7.01	6.17	0.86	7.03			
21	2.44	2.41	3.41	0.52	3.93			
28	1.88	2.16	2.35	0.45	2.80			

[a] MMI = Mount modification I.
[b] Measurement made with a battery-operated filter photometer.
[c] False-positive tests, see text for details.

centration value nearly midway between that for Cq and that for the sum of Cq and DECq.

When Haskins MMII was compared with HPLC determination of Cq (Table 2), the correlation data were $y=1.097x-1.3027$; $n=21$, $r=0.9895$ (Fig. 2). When the comparison was with the HPLC-determined sum of Cq+DECq, the corresponding data were $y=0.8415x-1.3078$; $n=21$, $r=0.9921$. With Haskins MMII, as with Haskins MMI, the sensitivity of the method to DECq is half that of Cq itself. This fact is reflected in the slope of the linear, least-squares correlation calculated to take this relationship into effect: $y=0.9538x-1.3309$; $n=21$, $r=0.9920$ where $x=[\text{Cq}]+\frac{1}{2}[\text{DECq}]$.

Specific-gravity-corrected and uncorrected values for Haskins MMII urine determinations for the three volunteers were compared with corresponding whole-blood Cq values from HPLC quantification of filter-paper blood spots. Comparison of the uncorrected Haskins MMII value with the whole-blood Cq concentration yielded the linear least-squares relationships $y=64.37x-1728$; $n=21$, $r=0.8272$ (Fig. 3). When the Haskins MMII values were adjusted to correct for dilution or concentration of urine by normalization of the sample specific gravity to the average specific gravity for each individual, the correlation was not significantly improved.

The TLC results for the samples of Tables 1 and 2 provided confirmation for the Haskins MMI and Haskins MMII tests; TLC determinations may be run in field laboratories which is usually not the case for HPLC analyses. The time=0 sample for volunteer

Table 2. Results of urine and blood tests on three volunteers after four doses of chloroquine diphosphate, at weekly intervals

Volunteer and sampling time (days)	Urine results (μmol/l)					Blood results (nmol/l)		
	Haskins (modified)		HPLC			HPLC		
	MMI[a]	MMII[a]	Cq	DEC-q	Cq + DECq	Cq	DECq	Cq + DECq
SW: 0	49.1	42.3	39.8	10.9	50.7	369	272	641
3	65.1	66.7	58.9	21.8	80.7	848	726	1574
7	14.5	14.1	17.3	6.50	23.8	438	313	751
10	23.1	22.8	19.6	7.22	26.8	357	261	618
14	34.4	33.9	32.6	9.80	42.4	301	227	528
21	9.80	9.89	8.23	3.44	11.7	148	132	280
28	6.98	5.73	4.91	2.89	7.80	105	86	191
CD: 0	26.1	25.2	24.0	8.91	32.9	257	213	470
3	24.1	27.5	21.8	6.50	28.3	504	489	992
7	11.3	9.39	11.1	3.06	14.2	266	224	490
10	13.5	11.1	11.1	3.96	15.1	216	186	402
14	5.00	2.72	3.69	1.58	5.27	117	137	254
21	5.29	4.19	3.79	1.58	5.37	93	89	182
28	3.57	3.00	2.35	1.34	3.69	64	54	118
FC: 0	26.1	24.6	23.3	3.92	27.2	435	162	597
3	50.0	39.8	41.9	9.49	51.4	717	304	1021
7	22.7	23.4	23.4	4.58	27.9	545	193	737
10	14.6	10.5	12.5	3.54	16.0	423	193	615
14	14.6	10.4	11.7	3.27	15.0	306	138	443
21	11.2	7.51	9.83	2.82	12.7	213	100	313
28	14.9	11.7	13.4	3.27	16.7	157	78	236

[a] MMI = Mount modification I; MMII = Mount modification II.

CD was seen to give a result of about 10 μmol/l by the Haskins MMI test. TLC assay demonstrated the absence of Cq and DECq in the sample. This example illustrates the occasional false-positive result that can occur with ion-pair extraction colorimetric methods and the utility of TLC in detecting such occurrences.

DISCUSSION

It is often desirable to determine the levels of antimalarial drugs in malaria field studies in order to identify persons who should be excluded from the study based on evidence of such drug use, to monitor chemoprophylaxis or treatment compliance, or to screen for possible resistance to the drug by the parasites. The procedures in this paper represent a family of methods to address these needs. The two modifications of the Haskins method permit the determination of urine levels of chloroquine even under primitive conditions since they do not require electricity. A hand-held, battery-operated filter photometer is used for quantification, and the defoamer allows separation of layers without centrifugation. Both colorimetric methods outlined in the present work are more sensitive than previously published methods for determining Cq (*3–5*, *8*, *9*). None of these methods, including the two described in the present work, however, is specific for Cq, since other basic compounds forming coloured ion-pairs partitioning into the organic layer can interfere. The TLC method can be used, if desired, to complement

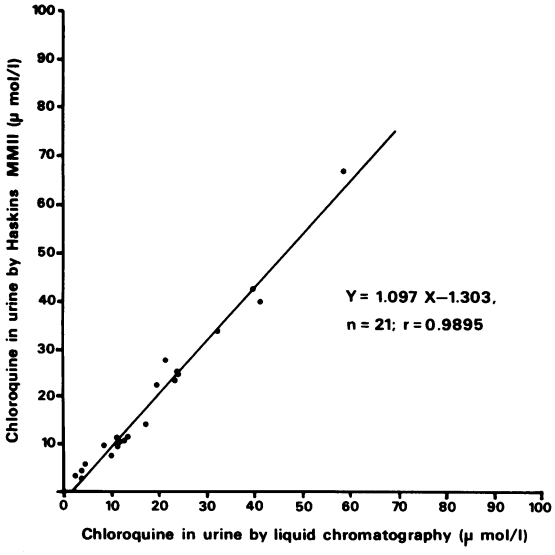

Fig. 2. Comparison of the Haskins MMII results with high-performance liquid chromatographic determination of chloroquine in urine using the data in Table 2. The Haskins MMII results represent a contribution of DECq as well as Cq (see text).

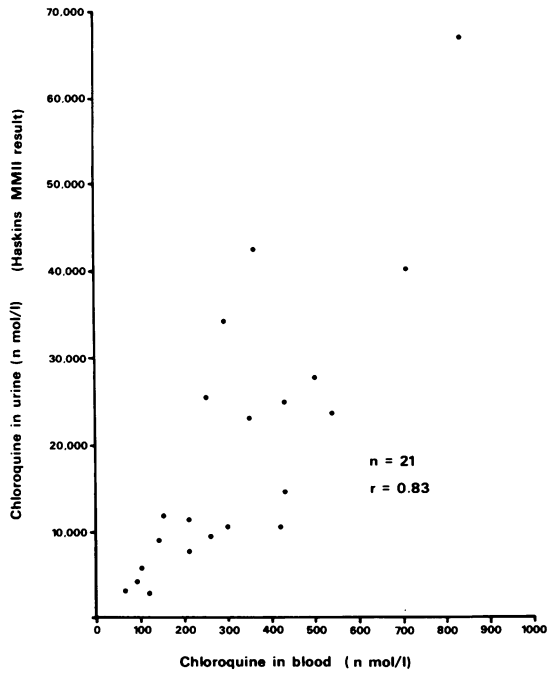

Fig. 3. Comparison of the Haskins MMII result and the concentration of chloroquine in corresponding blood samples. The Haskins MMII results represent a contribution of DECq as well as Cq (see text).

the colorimetric methods and provide confirmation of identity for Cq and DECq. High-performance, thin-layer chromatography (HPTLC) permits even more efficient separation of Cq and DECq from each other and from other, potentially interfering, basic drugs (Mount & Churchill, unpublished results).

Of the two modifications of the Haskins test described above, Haskins MMII is simpler and, although marginally less sensitive, will serve for most purposes. Haskins MMI has a lower limit of detection but requires more time and effort to perform. Chloroform may be substituted for dichloromethane, if desired, in the latter test.

Haskins MMI employs a step where MTBE solvent is transferred as completely as possible (for simplicity) but without aliquoting a specific volume. This step is rather reproducible, however, as reflected in the good correlation between results from the Haskins MMI and those from HPLC.

Based on the fact that DECq is detected with one-half the sensitivity of Cq for both modified Haskins colorimetric methods, correlations of colorimetric results with the sum [Cq]+½[DECq] found by HPLC is expected to yield a linear relationship with a slope near 1. Comparison of the Haskins MMI results with the HPLC results in Table 1 yields a slope of 0.89; the colorimetric result underestimates [Cq]+½[DECq] by about 11%. Comparison of the corresponding results in Table 2 shows a slope of 0.970, much nearer the expected value of 1.0. Similar comparison of Table 2 values for Haskins MMII and [Cq]+½[DECq], determined by HPLC, results in a slope of 0.95. The results from Table 2 indicate that the modified Haskins colorimetric methods in general yield values intermediate between [Cq] and the sum ([Cq]+[DECq]).

The Haskins MMII method may be applied directly to 2-ml urine samples containing from 0 to 30 μmol/l. The method is linear to a concentration of 100 μmol/l, although at levels above 30 μmol/l a dilution is required in the final step so that the photometer reading is on scale and in the region of acceptable photometric accuracy. Alternatively, if an initial test shows an absorbance reading that is at the high end of the scale, a smaller volume of urine may be taken for a subsequent determination and the result calculated accordingly. The linear range of the method could be increased by using higher concentrations of methyl

orange, but at the expense of a higher detection limit owing to an increased absorbance found for the blank. Field studies have shown that concentrations of Cq in urine of 3 μmol/l and 10 μmol/l can correspond to whole-blood concentrations of as much as 300 nmol/l and 600 nmol/l, respectively (Steketee, Churchill, & Mount, unpublished results). A negative test for Cq in urine by Haskins MMII indicates the presence of less than 100 ng/ml (300 nmol/l) of Cq in the blood.

The TLC method uses the initial extraction procedure of Haskins MMI, minimizing the variety of solutions and glassware needed in the field to apply all three methods.

The uncorrected values for Haskins MMII in Table 2 correlate well with blood levels of Cq. When the Haskins MMII urine values were corrected to reflect the degree of hydration for each individual (normalized to the average urine specific gravity for each individual), the correlation was not significantly improved. This might have been expected, since the degree of hydration of the laboratory personnel serving as volunteers was not expected to vary extensively and did not do so. Although field samples cannot be normalized relative to an average specific gravity for an individual, averages for populations or some other basis may be used to normalize drug concentrations to select the degree of hydration; correlations with blood concentrations may be determined to establish the advisability of such normalization procedures.

In comparing the two phases of the volunteer study (data found in Tables 1 and 2), it is clear that the whole-blood concentrations of Cq and DECq increase at corresponding sampling times, as expected, reflecting the accumulation and subsequent release of these compounds over time. The ratio of DECq metabolite to Cq is greater for samples in the second phase of the study than in the first. The correlation between Haskins MMII results and whole-blood Cq concentrations is high, as calculated from the data in Table 2. The extent of the correlation is probably enhanced by the fact that the three volunteers underwent the same chemoprophylactic regimen. A more general and realistic evaluation of the correlation of urine concentrations with whole-blood concentrations of Cq will require field data on a larger population with varying histories of chloroquine ingestion.

In summary, we feel the present work and other recent studies contribute effective methods for the field assay of urinary levels of chloroquine. The insensitivity and unreliability of the Dill–Glazko test has been demonstrated in several studies (*6–8*), and the three most commonly used tests for Cq (and metabolites) in urine have also been evaluated and compared (*8*). The method of Bergqvist et al. (*9*) is a substantial improvement over earlier tests; it can be used in remote locations where electricity is not available if modified to employ a hand-held, battery-operated filter photometer such as was used in the present study. The methods described here permit the quantification of Cq and, if desired, confirmation of the identity of Cq and its major metabolite DECq. The Haskins MMII method is most nearly comparable in its ease of use to the Bergvist method (*9*) and has a detection limit of 3 μmol/l (1 μg/ml), compared with 10 μmol/l (3 μg/ml) for the latter. Workers planning field studies may select the methods to be used depending on the information they need, the facilities available, and the time and effort they can give. The results of field application of the methods described in this paper will be published separately.

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RÉSUMÉ

DOSAGE SUR LE TERRAIN DE LA CHLOROQUINE ET DE SES MÉTABOLITES URINAIRES PAR COLORIMÉTRIE ET CHROMATOGRAPHIE EN COUCHE MINCE

Trois méthodes utilisables sur le terrain ont été élaborées et évaluées pour le dosage quantitatif d'un antipaludéen: la chloroquine (Cq). Deux de ces méthodes sont des modifications du test de Haskins fondées sur la mesure de

l'absorbance à 420 nm à l'aide d'un photomètre portatif à filtres fonctionnant sur batterie. La première (Haskins MMI) a été optimisée du point de vue de la sensibilité et sa limite de détection est de 1 μmol/l (0,3 μg/ml). La seconde (Haskins MMII), dont la limite de détection est de 3 μmol/l (1 μg/ml), a été optimisée en ce qui concerne la facilité d'utilisation. Dans les deux cas, la limite de détection du principal métabolite de la chloroquine, la déséthylchloroquine (DECq) est le double de la limite indiquée pour la substance mère. S'il y a lieu, la présence de Cq et de DECq peut être confirmée par une méthode de chromatographie sur couche mince (TLC) dont le principe est analogue.

Une étude a été effectuée sur des volontaires pour comparer ces méthodes avec la méthode de référence utilisée au laboratoire, qui fait appel à la chromatographie liquide haute performance (HPLC). Trois volontaires ont participé à l'étude qui s'est déroulée en deux phases. Dans la phase I, chaque volontaire a reçu un comprimé de diphosphate de chloroquine (300 mg de chloroquine base) et des échantillons d'urine et de sang ont été prélevés à différentes reprises sur une période de quatre semaines. La phase II a commencé à la fin de la quatrième semaine de la phase I. Les volontaires ont reçu quatre doses hebdomadaires (un comprimé par dose) et des échantillons de sang et d'urine ont été recueillis selon un calendrier analogue à celui de la phase I, avec la différence que les prélèvements de sang ont été plus nombreux. Les résultats photométriques se comparent bien avec la quantité [Cq] + ½[DECq] calculée à partir des résultats obtenus par HPLC. La comparaison des résultats de la méthode Haskins MMII avec la fonction [Cq] + ½[DECq] donne les paramètres de corrélation suivants: $y=0{,}954x-1{,}331$; $n=21$; $r=0{,}9920$ avec $x=[Cq]+½[DECq]$. On a également constaté une relation linéaire entre les concentrations urinaires obtenues par la méthode Haskins MMII (urine) et les concentrations correspondantes de Cq dans le sang. Le coefficient de corrélation calculé par la méthode des moindres carrés est égal à $r=0{,}8272$. La correction des concentrations urinaires en fonction de la densité de l'urine pour tenir compte de l'état d'hydratation des sujets n'a pas amélioré la corrélation de façon significative.

La méthode récemment publiée de Bergqvist et al. et les méthodes décrites dans le présent article constituent une amélioration importante par rapport aux méthodes précédemment utilisées sur le terrain pour évaluer la quantité de Cq présente dans l'urine, notamment celles de Dill-Glazko et de Wilson-Edeson et la méthode de Haskins non modifiée. La méthode de Dill-Glazko, largement utilisée en raison de sa simplicité, est beaucoup moins sensible que les autres et manque de fiabilité. Celle de Bergqvist et al. a une limite de détection de 10 μmol/l contre 3 μmol/l pour la méthode Haskins MMII. L'emploi d'un agent anti-mousse qui permet de séparer les solvants sans centrifugation dans la méthode Haskins MMI et d'un photomètre à filtres portatif fonctionnant sur batterie dans les deux méthodes MMI et MMII fait que ces méthodes sont vraiment utilisables sur le terrain. Si le même type de photomètre était utilisé avec la méthode de Bergqvist et al., celle-ci pourrait également être utilisée dans les zones dépourvues d'électricité.

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