Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1960, **22**, 663-668

A Field Test for the Estimation of Chloroquine in Urine*

GERHARD FUHRMANN, Sc.D.[1]

The use of antimalarial drugs is indissolubly linked with the use of insecticides in malaria eradication campaigns and is, indeed, of quite particular importance in areas in which insecticides have for various reasons—such as acquired resistance by anophelines—proved to be of limited value or in which residual spraying has ceased and potential foci of infection must be suppressed.

With the increasing use of chloroquine for mass prophylaxis it has become desirable to develop a reasonably simple technique for ensuring that the drug distributed is in fact taken regularly. The author describes a modification of a method first developed by him in 1950, which, although simpler than the original method, nevertheless permits the estimation of chloroquine in urine in amounts of 0.25-0.95 mg per 100 ml of urine. The equipment required, including a colorimeter needing no electrical current, is suitable for use in relatively primitive working conditions, and the test is designed for field application.

There are a number of methods for chloroquine estimation in urine, based on different principles. The first group derives from the formation of an organic complex of chloroquine with methyl orange (Brodie & Udenfriend, 1945; Haskins, 1958); another from the conversion of chloroquine to a fluorescent compound by ultraviolet irradiation (Brodie et al., 1947), and the last from the formation of an insoluble complex of chloroquine with certain inorganic acids and salts (Fuhrmann, 1950; Pereira & Paulini, 1956). Of the latter the most common is mercuric iodide, which, in combination with potassium iodide, forms a highly insoluble salt with chloroquine. The reagent, composed of mercuric iodide and potassium iodide in the proportion of approximately 1 : 4, is known as Mayer-Tanret's reagent.

All the above-mentioned methods are laboratory techniques and require relatively complicated apparatus, glass-ware and chemicals so that most of them are not suitable for routine application under field conditions.

This paper describes a modification of the method of Fuhrmann (1950), which, although considerably simpler than the original test, nevertheless permits the estimation of chloroquine in urine in amounts of 0.25 mg to 0.95 mg per 100 ml of urine.

* Paper submitted to the WHO Scientific Group on Malaria Research, November 1959

[1] Chief, Division of Clinical Chemistry, Institute for Tropical Diseases, Hamburg, Germany.

DESCRIPTION OF MODIFIED TEST

Principle

Chloroquine base is freed from urine by the addition of alkali and then extracted with gasolene (motor car petrol). The dissolved chloroquine is removed from the gasolene by shaking with diluted sulfuric acid to which Mayer-Tanret's reagent is then added. The turbidity produced is measured in the Polytest colorimeter (Zeiss-Ikon A.G.), using a grey wedge, and the amount of chloroquine in urine (in mg%) is read from a table or a calibrated standard curve. The reaction of chloroquine with Mayer-Tanret's reagent is not specific as substances such as proteins, quinine and other 4- or 8-substituted quinolines also react with this reagent. In most cases, however, the presence of these substances can be excluded, if necessary, by blank controls.

Supplies and apparatus

In designing the test one has to consider that the usual laboratory equipment, such as separating-funnels, is not always available under field conditions. The present method therefore uses simplified equipment. The number of the flasks etc. specified below depends on the number of chloroquine estimations carried out simultaneously and may be varied according to the demands. The following set of equipment is sufficient for 20-30 tests daily:

Volumetric flasks with ground glass stoppers:
1000 or 2000 ml (2-4)
250 ml (6-10)
100 ml (6-10).

Graduated cylinders:
1000 or 2000 ml (2-4; may be replaced by volumetric flasks of equal capacity, listed above)
100 ml (6-10)
25 ml (6-10).

Glass funnels about 10-cm diameter (6-10).

Folded filter paper, 9-cm diameter.

Glass or porcelain evaporating dishes, 6 cm diameter (6-10).

Pot or beaker (800-1000 ml), 10-15-cm diameter, for water-bath, on tripod, kerosene or spirit pressure burner (" Primus " type).

Pipettes:
20-ml pipettes (6-10)
5-ml pipettes (6-10)
1-ml graduated pipettes (1-2).

Zeiss Polytest colorimeter, equipped with grey wedge No. 123 with green filter and 2-cm plastic cuvettes (further described and illustrated below).

Test-tubes (12) with rack.

Stop-watch or alarm clock.

Reagents

(*a*) Ordinary motor car gasolene (no oil admixture).

(*b*) Bleaching clay as absorbing agent.

(*c*) 0.2 N sulfuric acid (prepared from 200 ml of normal sulfuric acid to which water is added up to 1000 ml).

(*d*) Mayer-Tanret's reagent. To 10 g of potassium iodide dissolved in 200 ml hot water, 2.7 g of mercuric iodide are added and dissolved under stirring. After cooling, the solution is stabilized by the addition of 2.5 ml of glacial acetic acid. The reagent must be kept cool, in a brown bottle. It remains unchanged for several weeks.

(*e*) Potassium hydroxyde, 33% aqueous solution.

(*f*) Methyl alcohol, absolute.

(*g*) Sulfosalycilic acid, 20% aqueous solution.

Note. The ordinary motor car gasolene is often coloured red by the addition of Sudan red and must be decolorized before use; otherwise the dye will pass into the final solution of sulfuric acid. For decolorization 1-2 spoonfuls of absorbant (bleaching clay, listed above) are added to 1-2 litres of gasolene in a volumetric flask, and thoroughly shaken for one minute, with occasional lifting of the stopper. The mixture is then filtered through a folded filter paper. Usually a single treatment is sufficient. If the filtered gasolene is still faintly yellow a second treatment will render it colourless.

Experiments to avoid the procedure of decolorization and to work with the original gasolene, taking it as the blank in the colorimeter, have failed. The part of Sudan red which passes into the sulfuric acid will also precipitate with Mayer-Tanret's reagent, thus yielding additional and elevated turbidity values.

Performance of test

First of all, the urine is examined to see if it is free of albumins; if it is not, the albumins have to be eliminated before the test is performed. To carry out this examination 3-5 drops of sulfosalicylic acid are added to a sample of about 4-5 ml of urine in a test tube. If albumin is present turbidity or precipitation will be observed. Chloroquine does not react with the sulfosalicylic acid. If there is albuminuria, the urine must be boiled for half to one minute. After cooling, the boiled urine is filtered through a folded filter paper into a graduated 100-ml cylinder up to the 100 mark. Albumin-free urine must also be filtered before shaking it with gasoline in order to prevent or diminish formation of emulsions.

Now, 100 ml of albumin-free and filtered urine are alkalinized by the addition of 5 ml of potassium hydroxyde in a 250-ml volumetric flask. The volume is made up to 250 ml with decolorized gasoline. The flask containing the mixture is shaken, with occasional lifting of the stopper. Very vigorous shaking is not necessary and should be avoided as it may cause the formation of emulsions which cannot easily be separated. Should an emulsion be formed, it can in most cases be cleared by the addition of 1-2 ml of methyl alcohol.

When the gasolene and urine have separated into two layers, 75 ml of the supernatant gasolene are carefully decanted into a graduated 100-ml cylinder, taking care that no urine is decanted. 0.2 N sulfuric acid is then added to bring the total volume to 100 ml. The two liquids are shaken again in a 250-ml volumetric flask. To facilitate the removal of sulfuric acid, the mixture is poured into a graduated 100-ml cylinder. This procedure is necessary if the 20-ml pipette cannot be dipped completely into the acid layer. After some time the gasolene (upper layer) and the sulfuric acid solution (lower layer) will have separated. Using the pipette, 20 ml of the underlying sulfuric acid are now removed; to do this, the pipette is closed with the finger at the top and then passed through the gasolene layer, ensuring that no gasolene penetrates into the pipette. The sulfuric acid solution is transferred into an evaporating dish and

heated for 2-5 minutes in the water-bath to eliminate any traces of gasolene. If this is not done, the gasolene may retain dissolved chloroquine. After cooling, the acid is poured into a graduated 25-ml cylinder, and the evaporated fluid is replaced by the addition of 0.2 N sulfuric acid, bringing the total volume to 20 ml.

Now 5 ml of the sulfuric acid solution are pipetted off into a test-tube and 0.1 ml of Mayer-Tanret's reagent is added. After 10 minutes the turbidity produced is measured in the Polytest colorimeter using the 2-cm cuvette and the grey wedge No. 123, according to the instructions for handling the colorimeter. If the turbidity values are too high the turbid solution should be diluted with 0.2 N sulfuric acid. The amount of chloroquine present in 100 ml of urine is read directly from Table 4 or the standard curve in Fig. 1 (these are given below). If the solution has been diluted the results must be corrected accordingly.

ASSAY OF VALIDITY OF TEST

Chloroquine excretion is given in mg per 100 ml of urine (mg%). This is not entirely correct as it is necessary, for estimation of the daily excretion of chloroquine, to examine a sample of the mixed urine collected over 24 hours. Previous assessments of chloroquine estimation in urine have shown that chloroquine, administered orally or intraveneously, is only partly excreted in the urine; the rest is reduced by the organism after absorption, and about 8% is also voided with the faeces. The reported amounts of chloroquine base excreted in urine vary, according to the reports of several authors, between 14% and 24% of the chloroquine administered (Berliner et al., 1948; Bruce-Chwatt, 1956; Fuhrmann & Koenig, 1955; Koenig & Fuhrmann, 1956; Jailer, Rosenfeld & Shannon, 1947). The amounts of chloroquine excreted in urine during ten days after the administration of a single dose of 300 mg of chloroquine base (=2 tablets of Resochin diphosphate) found by Fuhrmann & Koenig (1955) are shown in Table 1.

It is obvious that only the figures of total excretion during 24 hours present an exact picture of chloroquine excretion. As can be seen from Table 1, percentage excretion and excretion calculated per 100 ml of urine are by no means in conformity, since the level of total excretion is also dependent on the amount of urine produced. Under field conditions it is impossible in most cases to collect the urine quantitatively during 24 hours, and only one portion of urine (minimum quantity, 100 ml) can be examined. The figures of the third column of Table 1 may serve as a guide for the amounts of chloroquine excretion during each day following chloroquine administration.

TABLE 1
EXCRETION OF CHLOROQUINE IN MAN AFTER THE ADMINISTRATION OF A SINGLE DOSE OF 300 MG OF BASE

Day after administration	Daily excretion (%)	Mg per 100 ml of urine (mg%) [a]
1	5.9	1.20
2	2.5	0.90
3	1.8	0.65
4	1.4	0.60
5	1.7	0.50
6	1.3	0.50
7	1.2	0.50
8	1.1	0.40
9	1.1	0.35
10	0.8	0.30
Total excretion after 10 days	18.8	

[a] Mean values of 42 tests

We were interested to know whether there was any correlation between the figures for extraction of chloroquine with gasolene and those for extraction with ether. Aqueous standard solutions of chloroquine were used for this purpose. The results obtained are shown in Table 2.

The readings given in Table 2 were made with the Zeiss photometer Elko II and the Pulfrich photometer, using 1-cm layers and filter S 57, 10 minutes after the addition of the reagent. Each figure quoted in this table represents the mean of five readings. It appears from the table that results obtained with ether and with gasolene are very close. Repeated assays using the same concentrations showed that results were highly reproducible.

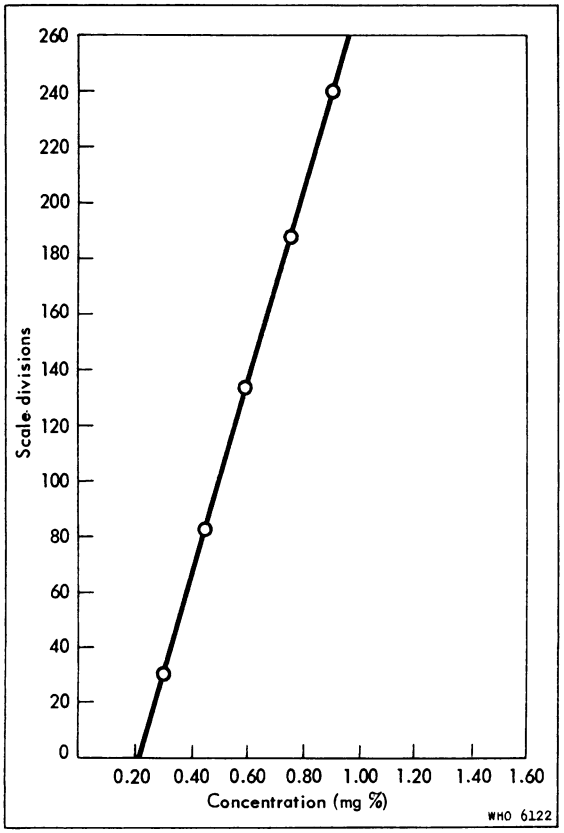
Following the procedure described above for performance of the test the values obtained from the photometric readings were related to the corresponding colorimetric values with the Polytest colorimeter, using grey wedge No. 123. The values obtained are shown graphically in Fig. 1. In conformity with Lambert-Beer's law the extinction is a linear function of the concentration. Values obtained from the curve of Fig. 1 are shown in Table 4. The grey wedge

TABLE 2
COMPARATIVE RESULTS OF CHLOROQUINE EXTRACTION FROM KNOWN AQUEOUS SOLUTIONS USING ETHER AND GASOLENE

Concentration (mg% of solution)	Ether [a]	Gasolene [a]
3.00	1.44	1.41
2.70	1.26	1.27
2.40	1.11	1.14
2.10	0.99	1.01
1.80	0.89	0.90
1.50	0.74	0.77
1.20	0.62	0.61
1.05	0.53	0.52
0.90	0.46	0.44
0.75	0.35	0.34
0.60	0.23	0.26
0.45	0.19	0.19
0.30	0.08	0.09

[a] The figures given for ether and gasolene represent extinction units of the photometer used.

FIG. 1
COLORIMETRIC VALUES OF CHLOROQUINE IN URINE



No. 123 with green filter was selected from a set of a number of other wedges as being the most suitable.

Following the establishment of this curve, known concentrations of chloroquine (mg%) were then added to urine samples and processed as indicated above. Table 3 shows the results obtained photometrically and colorimetrically. Table 3 also shows the amounts of chloroquine excreted in the urine of two test subjects who had orally taken two tablets of Resochin Bayer (300 mg of chloroquine base). It appears that there is good agreement between the results of the photometric and colorimetric methods of chloroquine assessment.

TABLE 3
CHLOROQUINE ASSAYS IN URINE, USING PHOTOMETRIC AND COLORIMETRIC (POLYTEST COLORIMETER) METHODS WITH FUHRMANN'S TEST

Chloroquine base added to urine samples			Comparison of photometric and colorimetric values for chloroquine base excreted in urine of two subjects	
Prepared concentration (mg%)	Photometric values (mg%)	Colorimetric values (mg%)	Photometric values (mg%)	Colorimetric values (mg%)
0.45	0.40	0.37	2.58	2.70[a]
0.45	0.44	0.46	2.08	1.70 [a]
0.45	0.30	0.43	1.10	1.18 [a]
0.50	0.40	0.54	0.26	0.42
0.50	0.52	0.64	1.52	1.54 [a]
0.75	0.68	0.64	0.40	0.52
0.75	0.68	0.55		
0.75	0.56	0.73		
0.75	0.68	0.75		
1.50	1.66	1.68 [a]		
1.50	1.52	1.55 [a]		
1.50	1.48	1.45 [a]		
1.50	1.50	1.50 [a]		
3.00	2.90	2.78 [a]		

[a] Obtained by aliquot dilution

POLYTEST COLORIMETER

As already stated, the instrument used for the estimation of the degree of turbidity obtained is the Zeiss Ikon Polytest Universal Colorimeter with

interchangeable wedge and green filter (Fig. 2). This instrument needs no electric current and is robust, simple, reliable for general work, portable and relatively cheap (about US $40 including wedge). It can be recommended for the estimation of chloroquine in urine in field conditions.

FIG. 2
ZEISS-IKON POLYTEST COLORIMETER

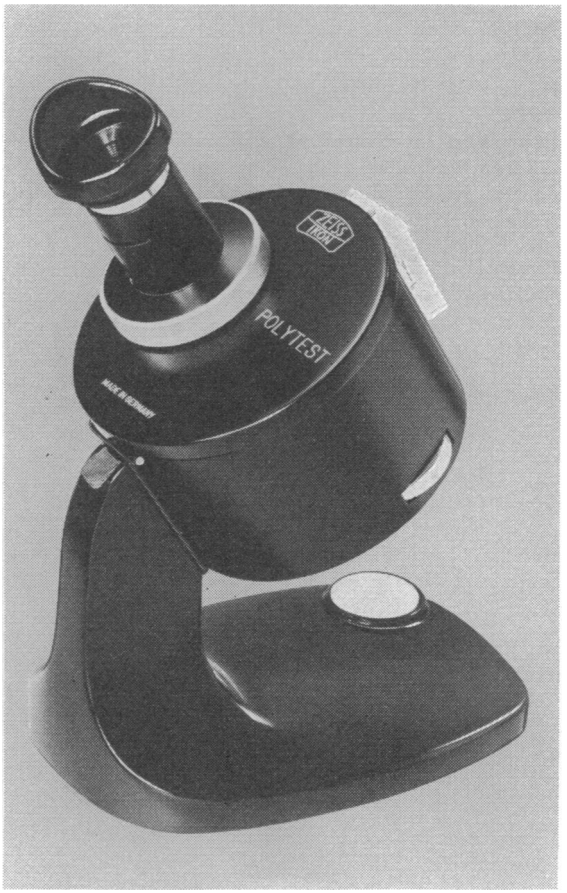


Table 4 has been prepared for the estimation of chloroquine in urine using the Polytest colorimeter. It is valid when the instrument is used with the grey wedge No. 123, with green filter. The layer of the tested sample is 2 cm; the readings were taken ten minutes after the addition of Mayer-Tanret reagent.

TABLE 4

TABLE FOR COLORIMETRIC DETERMINATION OF CHLOROQUINE IN URINE USING ZEISS POLYTEST COLORIMETER

Chloroquine concentration (mg%)	Scale divisions
0.25	13
0.30	30
0.35	47
0.45	82
0.50	99
0.55	117
0.60	135
0.70	169
0.75	187
0.80	205
0.85	223
0.95	258

RÉSUMÉ

La chimiothérapie du paludisme a repris une grande importance avec les campagnes d'éradication. Son efficacité dépend évidemment de l'absorption régulière des médicaments. Diverses méthodes de contrôle indirect de l'absorption de la chloroquine ont été proposées, pour mesurer la quantité résiduelle excrétée par l'urine, qui est un indice de la quantité ingérée. L'auteur décrit ici une modification simplifiant sa propre méthode et permettant de doser la chloroquine dans les conditions parfois précaires du travail sur le terrain.

Le principe de la méthode consiste à libérer de l'urine la chloroquine-base en ajoutant un alcali, et à l'extraire par de l'essence (qualité pour moteur automobile). Elle est ensuite déplacée de l'essence par de l'acide sulfurique auquel on ajoute le réactif de Mayer-Tanret (solution aqueuse d'iodure mercurique et d'iodure de potassium). Un complexe insoluble se forme, dont l'opacité est évaluée par un colorimètre « Polytest » qui n'exige pas de courant électrique. La teneur en chloroquine, en mg%, est lue d'après une tabelle ou une courbe standard. L'urine doit être débarrassée de l'albumine qu'elle peut contenir par chauffage de 30 secondes et filtrage. L'essence doit être décolorée par un absorbant et filtrée, si elle est colorée en rouge. L'auteur décrit en détail le cheminement de la méthode.

La quantité de chloroquine évaluée, d'après la moyenne de 42 essais, passe de 0,9 mg par 100 ml à 0,3 mg du 2e au 10e jour après l'administration. La méthode est donc valable dans les limites de la pratique. L'auteur souligne qu'il y a une bonne concordance entre les résultats de cette méthode et ceux des méthodes photométriques et qu'elle peut être recommandée pour le travail sur le terrain.

REFERENCES

Berliner, R. W. et al. (1948) *J. clin. Invest.*, **27**, 98

Brodie, B. B. & Udenfriend, S. (1945) *J. biol. Chem.*, **158**, 705

Brodie, B. B., Udenfriend, S., Dill, W. & Downing, G. (1947) *J. biol. Chem.*, **168**, 311

Bruce-Chwatt, L. J. (1956) *Bull. Wld Hlth Org.*, **15**, 852

Fuhrmann, G. (1950) *Z. Tropenmed. Parasit.*, **2**, 279

Fuhrmann, G. & Koenig, K. (1955) *Z. Tropenmed. Parasit.*, **6**, 431

Haskins, W. T. (1958) *Amer. J. trop. Med. Hyg.*, **7**, 199

Jailer, J. W., Rosenfeld, M. & Shannon, J. R. (1947)) *J. clin. Invest.*, **28**, 1168

Koenig, K. & Fuhrmann, G. (1956) In: *Medizin und Chemie*, Weinheim/Bergstrasse, Verlag Chemie, vol. 5, p. 174

Pereira, J. P. & Paulini, E. (1956) *Rev. bras. Malar.* **8**, 613