

# A simple urine test for sulfonamides

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*The lack of a simple and reliable test for sulfonamides has created a problem because of the increasing use of these drugs in the chemotherapy of malaria. This paper describes a modification of the Bratton-Marshall technique, for which the reagents are easily obtainable and which can be carried out in simply equipped primary health care laboratories.*

A prerequisite for the conduct of clinical trials of antimalarial drugs and the interpretation of *in vitro* and *in vivo* tests for determining the drug sensitivity of *Plasmodium falciparum* is a simple and reliable method of assessing whether a patient has received medication prior to the trial or the performance of the test. As such tests have to be conducted under field conditions, a simple urine test is preferable. Such a test does exist for 4-aminoquinolines (4) but the increasing use of sulfonamides, usually in combination with pyrimethamine, and the poor results often obtained by the lignin test for sulfonamides (3) necessitated the development of a more reliable test for these drugs.

Feigl & Anger in 1975 described a modification of the reliable Bratton-Marshall reaction for sulfonamides (1) and produced a very sensitive and simple spot test on filter paper, but the reagent used is neither readily available nor listed in the current chemical catalogues (2). This paper describes another simple modification of the Bratton-Marshall technique, for which the reagents are obtainable and which can be carried out in simply equipped peripheral laboratories.

## MATERIALS AND METHODS

### Reagents

Reagent 1: sodium nitrite solution, 0.03 mmol/l or 0.2 mg/100 ml or 0.2% (w/v) in distilled water.

Reagent 2: hydrochloric acid (concentrated).

Reagent 3: Bratton-Marshall solution (BM); dissolve 20 mg N-(1-naphthyl)-ethylene diamine dihydrochloride in 20 ml distilled water, adding 3 drops of concentrated hydrochloric acid.

Reagents 1 and 2 are stable at room temperature. Reagent 3 is stable for at least 2 months if kept at 5-10 °C in a brown bottle.

### Procedure

- (i) Pipette 1 ml of urine into a test tube.
- (ii) Add 1 drop of the sodium nitrite solution (reagent 1) and 2 drops of concentrated hydrochloric acid and mix well. Let it stand for 1 minute.
- (iii) Add 3 drops of BM (reagent 3) and mix.

## RESULTS

The evaluation of the test is equally simple:

— it is *positive* for free sulfadoxine and other diazotizable arylamines when the solution possesses a persistent purple colour. If the test is to be read spectrophotometrically, optimum absorbance is at 540 nm;

— the test is *negative* if the solution does not become purple or if the purple colour quickly disappears and the solution becomes greenish or brownish.

## DISCUSSION

The test has been evaluated in the laboratory and in the clinic where the urine of 150 patients taking Fansidar<sup>®</sup> (sulfadoxine and pyrimethamine) was tested for the presence of sulfonamide. It has been shown to give reliable results and to be sensitive down to a sulfonamide concentration of 16 μmol/l of urine (or 5 mg/l or 0.5 mg/%) ; the lower the sulfonamide content of the urine, the longer it takes for the colour reaction to develop (30 seconds to 30 minutes).

False positives are obtained with nitrazepam, clonazepam, flunitrazepam and other drugs which carry an aromatic primary amino group or a drug which is metabolized to such compounds.

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In the original Bratton-Marshall test, sulfamate was used to remove the excess nitrous acid between steps (ii) and (iii) of the procedure, but Wehrli (5) has suggested that this could be overlooked. We found that such removal was, indeed, essential; however,

extensive laboratory and field trials indicated that the excess nitrous acid was removed by urea which is always present in the urine at concentrations that were always many times greater than the concentrations of sulfamate originally used.

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### RÉSUMÉ

#### UN TEST SIMPLE DE DÉTECTION DES SULFAMIDES DANS L'URINE

Pour exécuter les essais cliniques des médicaments anti-paludiques et interpréter les épreuves *in vitro* et *in vivo* de détermination de la pharmacosensibilité de *Plasmodium falciparum*, une condition préalable est de disposer d'une méthode simple et fiable permettant d'estimer si un malade a reçu un traitement avant l'essai ou l'exécution du test. L'absence d'un tel test pour les sulfamides était devenue un problème en raison de l'utilisation croissante de ces médicaments. Une épreuve sur l'urine, fondée sur la technique de Bratton-Marshall, a été mise au point pour les sulfamides. Ce test nécessite trois réactifs: 1) une solution de nitrite de sodium (0,03 mmol/l ou 0,2 mg/100 ml dans de l'eau distillée), 2) de l'acide chlorhydrique (concentré), 3) de la solution de Bratton-Marshall (obtenue en dissolvant 2 mg de dichlorhydrate de N-(naphthyl-1)-éthylènediamine dans

20 ml d'eau distillée, et en ajoutant 3 gouttes d'acide chlorhydrique concentré). Ces réactifs sont faciles à se procurer à la différence de ceux qui étaient utilisés dans le test de Bratton-Marshall original. Le test lui-même est simple à exécuter et à évaluer et il peut être effectué dans des laboratoires périphériques ne disposant que d'un matériel simple. Il a été montré qu'il donne des résultats fiables et qu'il est sensible à une concentration de sulfamides aussi faible que 16  $\mu\text{mol/l}$  d'urine (ou 5 mg/l). Des résultats faussement positifs sont obtenus avec le nitrazépam, le clonazépam, le flunitrazépam et d'autres médicaments qui comportent un groupement aminé primaire aromatique ou des médicaments qui sont métabolisés en de tels composés.

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