A simple urine spot test for monitoring dapsone self-administration in leprosy treatment

Han Huikeshoven

Abstract

A simple urine spot test for monitoring patient compliance to dapsone self-administration in leprosy therapy was recommended by WHO but later abandoned. The present article describes some important improvements to the test, which is characterized by its validity and straightforwardness.

Daily self-administration of dapsone is an essential part of multidrug regimens recommended by a WHO Study Group for the treatment of leprosy (1). These regimens are minimal, and the Study Group has emphasized that "continuity, regularity, and completion of chemotherapy are the keys to the success of the proposed multidrug regimens" (1). In some respects, patient compliance in leprosy therapy is more important than ever before.

As early as 1966, a WHO Expert Committee on Leprosy proposed the use of a simple urine spot test to monitor dapsone self-administration by patients (2). The test had its basis in the observation made almost 40 years ago by Fennell that urine containing sulfonamides undergoes the classical Ehrlich reaction with aldehydes to produce a brilliant yellow solution (3). This test for sulfonamides in urine has the advantage that it requires as reagent only an acidified solution of p-dimethylaminobenzaldehyde instead of the three reagents needed for the Bratton and Marshall test. In 1961, De Mello (4) described a technique of impregnating filter-paper with Ehrlich's reagent modified by Fennell for the detection of sulfonamides or sulfones in urine. Later, the test was further improved by De Castro et al. (5), and, in this version, a drop of urine that contains sulfones produces an orange spot on impregnated filter-paper.

The test was mentioned in an unpublished guide to leprosy control circulated by the World Health Organization in 1973 but it was omitted from the 1977 report of the WHO Expert Committee on Leprosy (6); instead a plea was made for "simpler methods for analysing urine for dapsone content". Reservations on the spot test probably arose because of its low sensitivity when test urine samples were compared with a standard aqueous solution containing 5 mg/l dapsone, as reported by Ellard et al. (7); these researchers proposed that the spot test be replaced by a quantitative colorimetric method of determining the ratio of the concentrations of dapsone and creatinine in urine, the inclusion of the concentration of creatinine compensating for variations in diuresis. Notwithstanding the many merits of the colorimetric method, there was still a need for a simple test for use in field work. A guide to leprosy control, published by WHO in 1980 (8), tentatively recommended a spot test employing four filter-papers, three of which are impregnated with one each of the reagents for the Bratton and Marshall reaction. The fourth paper is soaked in the urine to be investigated, and the three impregnated papers are then applied to it in succession. Instead of a test requiring only one filter-paper, this publication recommended one using four filter-papers and the same reagents rejected almost 40 years previously by Fennell in his search for a simple method.

In 1983, the results of a comparative study indicated, however, that the original spot test employing Ehrlich's reagent is far more straightforward to perform (9). Furthermore, in a recent evaluation of the original spot test involving 14 leprosy control centres in 12 countries, 1150 urine samples were examined (10); 11 participants found that it was easy to perform and practical for use in field work. However, comparison of the results using haemagglutination tests indicated that there were a high percentage of false negative results in one centre. Investigation of this indicated that the urine involved had a pH of ≥9 caused by growth of bacteria in the containers, which had been stored for 15 days before the urine was tested. Addition of a drop of acid changed the false negative spots into positives. On the
other hand, false positive spots due to cross-reacting sulfonamides faded away when acid was added. When all false outcomes were thus corrected, there was consonance between the results of haemagglutination and spot tests for 1109 of the 1150 urine specimens tested (96%). Furthermore, if a solution of dapsone in urine instead of in water is used as the standard, the results of the spot test are less ambiguous. Also, use of 50–70% ethanol, instead of absolute ethanol, completely dissolves the sodium dodecylbenzenesulfonate constituent of the reagent, producing brighter spots.

In a further study involving 20 volunteers (12), the sensitivity of the test, modified as described here, was examined. The results indicate that spot tests are, on average, negative when a patient misses three doses of dapsone; no negative spots can be expected in fully compliant patients who take 100 mg of dapsone daily, and no positive spot tests can be expected in patients who omit to take dapsone for \( \geq 1 \) week before the test. In the light of the literature on dapsone, this implies that all positive spot tests correspond to blood levels of dapsone well above the minimum inhibitory concentration (11) for preventing the growth of *Mycobacterium leprae*. In contrast, a negative spot test indicates that dapsone intake is less than that prescribed in the regimen and, possibly, that the time elapsed from the last intake is long enough to depress the blood level below the minimum inhibitory concentration.

The revised method for the spot test is fully described in Annex 1.

REFERENCES


RÉSUMÉ

UNE ÉPREUVE URINAIRE SIMPLE SUR PAPIER FILTRE POUR SURVEILLER L'AUTO-ADMINISTRATION DE DAPSONE DANS LE TRAITEMENT DE LA LÈPROME

Dès 1966, un Comité OMS d'experts de la Lèpre avait proposé l'utilisation d'une épreuve urinaire sur papier filtre pour surveiller si l'auto-administration de dapsone est bien observée. Dans cette épreuve simple, une goutte d'une urine contenant des sulfones produisait une tache orange sur un papier filtre imprégné d'un réactif d'Ehrlich modifié. Toutefois, dans ses publications ultérieures, l'OMS avait abandonné cette épreuve et avait proposé de recommander une épreuve urinaire utilisant quatre papiers filtres. Le présent article fait le point des réserves à l'égard de la première épreuve, plus simple, et décrit quelques améliorations importantes à apporter à la deuxième épreuve. La méthode révisée, décrite en détail dans une annexe, se caractérise par la validité de ses résultats et par sa simplicité d'exécution.
Annex 1

Revised method for the urine spot test for dapsone

Reagent

- p-Dimethylaminobenzaldehyde 0.2 g
- Oxalic acid 1.0 g
- Sodium dodecylbenzene-sulfonate 0.1 g

The above constituents are dissolved in 100 ml of 50-70% (v/v) ethanol. If stored in the dark, this solution is stable for 1 month.

Impregnation of filter-paper

Thick strips of filter-paper (Whatman No. 3) are soaked in a solution of the reagent and dried in air, preferably in the dark. If stored in the dark, the impregnated paper is stable for a few months depending on the local humidity.

Controls

An approximately 1 mol/l solution of hydrochloric acid is made up by diluting 10 ml of concentrated hydrochloric acid with 100 ml of water. A negative control solution (A) is then prepared by adding 10 ml of 1 mol/l hydrochloric acid to 90 ml of urine from an individual who has not taken dapsone or sulfonamides. Another solution (B) is then made up by dissolving 1 g dapsone in 100 ml of 1 mol/l hydrochloric acid, and 1 ml of this solution (B) is added to 49 ml of water to give solution (C). Finally, 1 ml of solution (C) is added to 39 ml of solution (A) to give solution (D), the positive control, which contains approximately 5 mg/l dapsone in acidified urine. The control solutions are stable for a month if kept at 4 °C.

Spot test

A drop of urine is placed on the impregnated filter-paper. After 1 minute, a yellow ring develops at the periphery, caused by urea, and an inner orange spot appears if dapsone is present. Add one drop of a solution of 0.5-1.0 mol/l hydrochloric acid to the spot: false negatives (in samples of urine with relatively high pH) become positives, and false positives (caused by presence of sulfonamides) fade away. The urine spots are best examined by viewing the paper against the light. Urine is positive if it gives a central spot whose intensity is equal to or greater than that produced by the positive control (D). In a borderline case, a spot is taken to be positive, particularly if the urine specimen is rather colourless. Solutions (A) and (D) can also be used to check the quality of the test paper: there should be a distinct difference between the spots made with solutions (A) and (D).

Interpretation

A positive test corresponds to a sulfone level in the blood well above the minimal inhibitory concentration for the multiplication of Mycobacterium leprae. A negative test indicates that dapsone was not taken according to the schedule of 100 mg per day and, possibly, that the time since the last intake is sufficiently long for the level of dapsone in blood to have fallen below the minimum inhibitory concentration.

---

b Methanol (100%) is a good alternative; use water if neither ethanol nor methanol is available, although p-dimethylaminobenzaldehyde is only sparingly soluble in water.