

Evaluation of a nitrite test kit (Stat-test) for the detection of significant bacteriuria

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SYNOPSIS A commercially marketed nitrite test kit (Stat-test) for the rapid detection of significant bacteriuria has been evaluated and found to have failed to detect 50% of all cases of significant bacteriuria and 46% of significant bacteriuria associated with nitrate-reducing organisms. The number of false positive results are negligible. The Stat-test, used in its present form, cannot be recommended as a satisfactory screening test for significant bacteriuria.

Enterobacteriaceae are the most prevalent organisms found in infected urine. Almost all enterobacteriaceae reduce nitrate, normally present in urine, to nitrite during active growth phase. The presence of nitrite can be readily detected by a simple diazotization reaction, the Griess test which was first described in 1879 and had been used extensively for testing the purity of water supplies. The reaction consists of the development of a red colour within seconds of the addition of an acidic alpha naphthylamine-sulphonilic acid reagent. The red coloured product, azo-alpha-aminonaphthalene-parabenzene sulphonic acid, is a relatively stable compound, the colour persisting for several hours (Schaus, 1956).

The introduction of the Griess test as an aid in the detection of significant bacteriuria (the presence of 100,000 or more organisms per millilitre of urine) has met with a mixed reception (Kahler and Guze, 1957; Smith, Thayer, Malta, and Utz, 1961; Sleight, 1965). One drawback of the Griess test has been the instability of the reagent, thus necessitating the frequent preparation of fresh solutions. To overcome this problem, a convenient and stable individual package of the Griess reagent has been developed and marketed by Mallickrodt Chemical Works (St. Louis, U.S.A. and Montreal, Canada) under the name of Stat-test.

MATERIALS AND METHODS

This study set out to determine if the urine samples of 100 normal individuals contained nitrite demonstrable by the Stat-test, and to evaluate the efficacy of the Stat-test in the detection of significant bacteriuria on 500 urine samples taken at random from patients in the wards and outpatient departments, comparing the results

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with those obtained by another chemical screening test the catalase test.

The specimens tested were either clean-voided, mid-stream, first morning urine samples or samples of urine passed during the day when at least four hours had elapsed after a previous voiding. The methods of urine collection, bacterial colony count, and the catalase test have been described elsewhere (Lie, 1967).

The Stat-test kit is individually packed in airtight foil. Each unit consists of a pliable plastic tube enclosed at one end with a cotton plug which acts as the reaction site for the test. Inside the plastic tube is a thin glass ampoule containing a stabilized solution of alpha-naphthylamine and sulphonilic acid. To perform the test, the end of the tube with the cotton plug is dipped into the urine specimen, the test unit is then removed from the specimen and the plastic tube squeezed to break the glass ampoule. This permits the reagent to come into contact with the urine-saturated cotton plug. According to the manufacturer, concentration of nitrite as low as one microgram per millilitre of urine will cause a pink to red colour to appear on the cotton plug within 10 seconds after release of the reagent. One microgram of nitrite is the amount estimated to be produced by 100,000 nitrate-reducing organisms (Kahler and Guze, 1957).

In this study, both the Stat-test and the catalase test were performed before all other examinations of the urine, that is, without prior knowledge of the results of microscopy and bacterial colony count.

RESULTS

None of the 100 urine samples from normal individuals (52 males and 48 females) gave a positive reaction to the Stat-test.

Among the 500 urine samples from patients, only 79 specimens showed significant bacteriuria as determined by the colony counts. The results of the Stat-test and the catalase test are summarized in Table I.

TABLE I

COMPARISON OF COLONY COUNTS AND THE RESULTS OF THE STAT-TEST AND THE CATALASE TEST

| Colony Counts | Number of Specimens | Stat-test | | Catalase Test | |
|-------------------------------------|---------------------|-----------|-------------|---------------|-----------|
| | | Positive | Negative | Positive | Negative |
| Greater than 100,000 per millilitre | 79 | 40 (50%) | 39 (50%) | 71 (90%) | 8 (10%) |
| Less than 100,000 per millilitre | 421 | 2 (0.5%) | 419 (99.5%) | 59 (14%) | 362 (86%) |
| Total | 500 | 42 | 458 | 130 | 370 |

While the Stat-test rarely gave a false positive reaction in urine samples with a colony count of less than 100,000 per millilitre, it failed to detect about 50% of significant bacteriuria. Even after the exclusion of instances of significant bacteriuria caused by non-nitrate-reducing organisms, there remained some 46% of significant bacteriuria giving a false negative reaction to the Stat-test (Table II).

TABLE II

CORRELATION OF POSITIVE REACTIONS TO THE STAT-TEST WITH THE BACTERIAL POPULATION OF SIGNIFICANT BACTERIURIA

| Organisms | Number of Specimens | Stat-test | |
|-------------------------------|---------------------|-----------|----------|
| | | Positive | Negative |
| <i>Nitrate-reducing</i> | | | |
| <i>Escherichia coli</i> | 39 | 22 | 17 |
| <i>Klebsiella-Aerobacter</i> | 18 | 12 | 6 |
| <i>Proteus species</i> | 13 | 5 | 8 |
| <i>Pseudomonas species</i> | 2 | 1 | 1 |
| <i>Paracolon</i> | 1 | 0 | 1 |
| <i>Staphylococcus albus</i> | 1 | 0 | 1 |
| Total | 74 | 40 (54%) | 34 (46%) |
| <i>Non-nitrate-reducing</i> | | | |
| <i>Streptococcus faecalis</i> | 3 | 0 | 3 |
| <i>Streptococcus viridans</i> | 1 | 0 | 1 |
| <i>Candida albicans</i> | 1 | 0 | 1 |
| Total | 5 | 0 | 5 |

DISCUSSION

The results of this study supported the accepted notion that uninfected urine contains no nitrite (Schaus, 1956). While the Stat-test was a superior form of the Griess test for the detection of nitrite by a diazotization reaction, it was unsatisfactory when used as a screening test for significant bacteriuria in our hands. Although the number of false positive reactions in the 421 urine specimens without significant bacteriuria was negligible (0.5%), the Stat-test had failed to detect about half the specimens with significant bacteriuria. In this respect the Stat-test was inferior to the catalase test which was positive in 90% of significant bacteriuria (Table I). The results of the catalase test incidentally corroborated those of an earlier study on a much larger number of urine specimens (Lie, 1967).

In published reports, the success of the Griess test, using self-prepared reagent, in detecting significant

bacteriuria has varied from 40 to 80% (Sleigh, 1965). Of the few reports available concerning the use of the Stat-test, both good, 82% (Walsh, Hildebrandt, and Prystowsky, 1966), and bad, 35% (Branson, 1966) correlations of a positive reaction to the Stat-test with significant bacteriuria have also been given. Thus the disappointing 50% failure rate of the Stat-test in detecting significant bacteriuria in our hands is not an isolated finding.

It is evident from Table II that the presence of non-nitrate-reducing organisms was not the cause of failure, since *Streptococcus*, *Candida* (and occasionally *Neisseria* and *Mycobacterium*) constituted less than 10% of the bacterial population of significant bacteriuria. Other possible reasons for the failure of the Griess test mentioned by Schaus (1956) are insufficient or no nitrate being present in the urine to act as a substrate; voiding so frequently that bacteria lack the necessary time to reduce nitrate; inhibition of the metabolic activity of the bacteria by antimicrobial therapy or urinary acidification; and the nitrite produced being further decomposed by the same bacteria.

Various modifications of the Griess test have been proposed. By incubating the urine, to which nitrate has been added, in a water bath at 37°C for four to six hours before testing for nitrites, Sleigh (1965) claims to have vastly improved the reliability of the Griess test. The same modification may be applied to the Stat-test in order to achieve better results. The advantages of speed and simplicity would then be lost, and with these the appeal of the Stat-test as a screening test for significant bacteriuria.

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