Evaluation of a new sensitive nitrite test as a reliable screening tool for bacteriuria

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SUMMARY A new sensitive nitrite test, the SRN test, was evaluated for its suitability as a reliable screening technique for the detection of bacteriuria. The SRN test was compared to a currently available nitrite test, the Microstix nitrite test, and the results obtained with both nitrite tests were assessed in comparison with the results of the quantitative culture method. Of 158 cases of significant bacteriuria found among 1060 randomly collected specimens, the SRN test detected 90% and the Microstix nitrite test, 30%. The higher reliability of the SRN test reflects its high capability of nitrite detection (≥ 0.1 ppm), and its ability to overcome interference by various factors, such as dark colour of the urine, presence of phenazopyridine, urobilinogen, blood, high concentration of ascorbate, and high urinary pH, all of which do interfere with the Microstix nitrite test. The high sensitivity of the SRN test allows detection of bacteriuria in urine specimens collected at random throughout the day; the test is therefore not restricted to the use of first-morning samples as are other nitrite tests. Since the SRN test was found to give a quantitative indication of the size of the bacterial population, the possibility of its use as an exact quantitative test under controlled conditions is discussed.

The quantitative urine culture is the most reliable way of diagnosing urinary tract infection (UTI). However, the high cost, the need for trained personnel, the inconvenience of collecting clean-voided specimens, and especially the need to treat the symptomatic patient immediately limit its routine use in office practice.

A simple screening test for bacteriuria, if rapid, accurate, and inexpensive would be of great advantage in the routine diagnosis of UTI. In addition, the availability of such a test would encourage screening programmes for asymptomatic populations with a high risk of developing UTI, such as pregnant women, diabetics, children, premature infants, and patients with catheterisation of the urinary tract. A number of rapid screening tests—culture procedures, chemical tests and combinations of the two methods—have recently become available. Of the chemical methods, the nitrite test appears to be the most commonly used, and it has recently appeared on the market in a variety of forms.

The nitrite tests are based on the ability of enteric Gram-negative bacteria, the commonest pathogens of the urinary tract, to reduce dietary-derived nitrate in the urine to nitrite. These tests, however, have often been reported to be inaccurate or unreliable. In this study, we evaluated a new sensitive nitrite test, the SRN test, for its suitability as an efficient screening test for detecting bacteriuria. Our study was thus designed to investigate the reliability and sensitivity of the test and the effect of various factors known to interfere with the accuracy of nitrite tests. The results obtained with the SRN test for a series of randomly collected urine specimens were compared with those given by a commonly used nitrite test, the Microstix nitrite test. The quantitative culture method was used as the control. Factors which can affect the accuracy of the SRN test as a technique for detecting bacteriuria, both qualitatively and quantitatively, are discussed.

Material and methods

Urine specimens

Urine specimens were collected at random from a general population of patients attending one of the health centres of the “Sick Fund of the General Labor Federation.” The samples were supplied to us by the bacteriology laboratory of this health centre. Each urine specimen was first tested for nitrite and

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then plated for culture (control). The pH and the nitrate concentration of each urine sample were also recorded.

**NITRITE TESTS**

Urine specimens were tested quantitatively for nitrite by the SRN test (Advanced Products, Beer-Sheva Ltd). The tests were carried out in two series of experiments: series I (443 specimens) was conducted in 1977 and series II (617 specimens) in 1978 (Table 1). In the presence of nitrite, a characteristic pink-violet colour develops, its intensity being proportional to the nitrite concentration. The quantitative determination of the nitrite content of each sample was carried out visually by comparison with a standard colour chart supplied with the test.

For purposes of comparison, the urine samples were also tested for nitrite by the nitrite pad area of the Microstix test (Ames Co, Division of Miles Laboratories, Stoke Poges, England), thereafter referred to as the “Microstix test.” According to package instructions the test is not quantitative, and any shade of pink appearing in the nitrite pad of the Microstix, was taken as a positive result.

The sensitivities of the SRN and Microstix tests were also compared as follows: urine samples, obtained from healthy men, to which increasing amounts of nitrites had been added were tested with both nitrite tests. The tests were carried out on both light and dark coloured urine so that the effect of the natural colour of the urine on the detection ability of the tests could be evaluated.

Possible interference factors which may be present in the urine were tested for their effect on the SRN test: nitrite-free urine samples with macroscopic haematuria or containing phenazopyridine, bilirubin, abnormally high concentration of urobilinogen (4-12 mg/dl), or ascorbic acid (10-100 mg/dl), to which nitrite had been added were tested with the SRN test, and its detection limit was determined.

The effect of urinary pH on the sensitivity of both nitrite tests was studied in nitrite-free urine specimens, obtained from healthy persons. These specimens had been adjusted to various pH levels ranging from 4 to 9. Various amounts of nitrite were added to each sample and the detection limits of the SRN and Microstix tests were determined.

**URINE CULTURES**

The conventional urine culture was used as the reference method. A 0.1 ml sample of each original specimen and 0.1 ml aliquots of serial dilutions of the specimen in sterile saline were plated on MacConkey’s agar. Colonies were counted after an overnight incubation at 37°C. Urine specimens with $\geq 10^5$ bacteria/ml were considered as showing significant bacteriuria. Pathogens in the bacteriuric specimens were identified as a routine procedure by the Central Laboratory of the General Labor Federation Clinic.

**URINARY NITRATE**

Urinary nitrate was determined quantitatively by the Szechrome NAS method: a 0.5 ml urine sample (containing 2 to 20 ppm NO$_3^-$) was mixed with 5 ml of the NAS reagent. The violet colour which develops is stable for at least an hour and the nitrate concentration is determined colorimetrically ($\lambda$ max 570 nm).

**URINARY pH**

The pH of the urine was determined with a PHM 62 standard pH meter.

**Results**

Of the 158 samples with significant bacteriuria 143 cases were detected with the SRN test (sensitivity of 90%), whereas only 50 cases were discerned by the Microstix test (sensitivity of 26 to 34%). The specificity of the SRN test was 99.3-99.5% (three false-positives—that is, positive nitrite assay in urine with $< 10^9$ bacteria/ml—in each of the series) as opposed to 99.9% for the Microstix test. The nitrite test was consistently negative at bacterial concentrations $< 10^6$ bacteria/ml.

Ninety-seven per cent of the bacteriuric cases were caused by enteric Gram-negative bacilli—that is, nitrate-reducing organisms (Table 2). The ability of the SRN test to detect bacteriuria in these cases was higher than that of the Microstix both for $10^5$ bacteria/ml and for higher bacterial concentrations, irrespective of the bacterial species. Three per cent of the bacteriuric specimens in the study contained Gram-positive cocci; this figure is in keeping with that usually found in the general population. In these cases, bacteriuria was not discerned by either of the two nitrite tests, since these organisms do not reduce nitrate.

The relation between the frequency of false-negatives in the nitrate-reducing bacteriuric specimens and the size of bacterial population (Table 3) shows that with the SRN test false-negatives were found only at the threshold concentration of significant bacteriuria. At higher bacterial concentrations the SRN test did not "miss" any case of bacteriuria. The Microstix test, however, did not discern any of the bacteriuric cases with $10^6$ bacteria/ml and failed to detect some cases at the higher bacterial concentrations, even at $\geq 10^9$ bacteria/ml.

The sensitivities of the SRN and Microstix tests.
Evaluation of a new sensitive nitrite test as a reliable screening tool for bacteriuria

Table 1  Ability of SRN test and Microstix test to detect significant bacteriuria in random urine specimens

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total no of bacteriuric specimens</th>
<th>Bacteria/ml*</th>
<th>No of specimens</th>
<th>No detected by nitrite test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SRN test</td>
</tr>
<tr>
<td>E. coli</td>
<td>132</td>
<td></td>
<td>10^6</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt; 10^8</td>
<td>97</td>
</tr>
<tr>
<td>Proteus spp</td>
<td>12</td>
<td></td>
<td>10^8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt; 10^8</td>
<td>6</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>8</td>
<td></td>
<td>10^6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt; 10^8</td>
<td>4</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>4</td>
<td></td>
<td>10^8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt; 10^8</td>
<td>3</td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>2</td>
<td></td>
<td>10^8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt; 10^8</td>
<td>1</td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>5</td>
<td></td>
<td>10^8</td>
<td>5</td>
</tr>
</tbody>
</table>

Bacterial concentration is given only in orders of magnitude.
*Culture method.

Table 2  Dependence of reactions of SRN and Microstix tests on bacterial species and bacterial concentrations in randomly collected bacteriuric specimens

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total no of bacteriuric specimens</th>
<th>Bacteria/ml*</th>
<th>% sensitivity†</th>
<th>Microstix test §</th>
<th>% specificity¶</th>
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<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>132</td>
<td></td>
<td>10^6</td>
<td>45</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt; 10^8</td>
<td>90</td>
<td>37</td>
</tr>
<tr>
<td>Proteus spp</td>
<td>12</td>
<td></td>
<td>10^8</td>
<td>90</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt; 10^8</td>
<td>91</td>
<td>34</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>8</td>
<td></td>
<td>10^8</td>
<td>13</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt; 10^8</td>
<td>37</td>
<td>34</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>4</td>
<td></td>
<td>10^8</td>
<td>37</td>
<td>34</td>
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<tr>
<td></td>
<td></td>
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<td>&gt; 10^8</td>
<td>90</td>
<td>34</td>
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<tr>
<td>Pseudomonas spp</td>
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<td>10^8</td>
<td>37</td>
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<td></td>
<td></td>
<td></td>
<td>&gt; 10^8</td>
<td>90</td>
<td>34</td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>5</td>
<td></td>
<td>10^8</td>
<td>90</td>
<td>34</td>
</tr>
</tbody>
</table>

Bacterial concentration is given only in orders of magnitude.
*Culture method.

Table 3  Ability of the SRN and Microstix tests to detect significant bacteriuria in random urine specimens with various concentrations of nitrate-reducing bacteria

<table>
<thead>
<tr>
<th>Bacterial concentration per ml</th>
<th>10^6</th>
<th>10^8</th>
<th>10^9</th>
<th>10^10</th>
<th>≥ 10^10</th>
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<tr>
<td>Series I</td>
<td>20</td>
<td>1</td>
<td>11</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>No detected—SRN test</td>
<td>15</td>
<td>1</td>
<td>11</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>No detected—Microstix test</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Series II</td>
<td>30</td>
<td>17</td>
<td>40</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>No of bacteriuric samples*</td>
<td>20</td>
<td>17</td>
<td>40</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>No detected—SRN test</td>
<td>0</td>
<td>6</td>
<td>23</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

Bacterial concentration is given only in orders of magnitude.
Microstix = nitrite pad of Microstix test.
*Culture method.

were also compared in an experimental system, as described above. Table 4 presents the colour intensities obtained with the SRN test and Microstix test in both light and dark nitrite-free urine with increasing amounts of added nitrites. It can be seen that the threshold level of SRN test is at least 40 times lower than that of the Microstix test. Furthermore, whereas the SRN test is not affected by the colour of the urine, natural urine colour does interfere with the Microstix test, which is less sensitive in darker urine samples.

An orange colour, present in the urine of patients treated with phenazopyridine does not interfere with the SRN test, but does affect the Microstix test as was also reported by Craig and Moffat. In six orange-coloured urine samples obtained from bacteriuric patients treated with phenazopyridine, concentrations of nitrites ranging from 1 to 20 ppm were detected by the SRN test. On the other hand, the Microstix test failed to detect nitrites in any of these bacteriuric specimens. Tests performed with both nitrite tests on bacteria-free urine coloured orange by
phenazopyridine, to which increasing amounts of nitrite had been added, showed that even concentrations as high as 40 ppm of nitrite could not be discerned by the Microstix test, as the pinkish orange colour obtained with these samples was almost indistinguishable from the orange colour of the control. On the other hand, the threshold level of detection of the SRN test in these samples was 0.1 ppm.

Since the presence in the urine of blood, bilirubin, an abnormally high concentration of urobilinogen, or a high concentration of ascorbic acid, has been reported to interfere with the Microstix or other nitrite tests, we examined the effect of these factors on the SRN test. In nitrite-free urine specimens with abnormally high urobilinogen concentration (4-12 mg/dl), bilirubin, macroscopic haematuria, or ascorbic acid (10-100 mg/dl) the detection limit of the SRN test was not affected and remained 0.1 ppm of nitrite.

The effect of pH of the urine on both nitrite tests was also determined (Fig. 1). It was found that the sensitivity of the SRN test was not affected by the pH of the urine, for both dark and light urine samples. On the other hand, the sensitivity of the Microstix test decreased significantly as the pH of the urine increased. Furthermore, for the Microstix test, interference of pH appears to be potentiated by the colour of the urine, the influence of the pH being greater in the darker samples.

In some bacteriuric specimens, a combination between various interfering factors such as pH, colour of the urine and other unidentified interfering factors may have given rise to a further suppression of the detection ability of the Microstix test. For example, the Microstix test failed to detect concentrations of nitrates as high as 20, 40 and even 100 ppm as detected by the SRN test. On the other hand, full recovery of 10, 20, and 40 ppm of additional nitrates added to these urine specimens was obtained with the SRN test.

As the SRN test gives quantitative results, it can be used as a measure of the size of the bacterial population. The relation between the nitrite concentration, as estimated by the SRN test, and the size of the bacterial population in randomly collected
bacteriuric specimens is presented in Fig. 2. Although there is some scattering of nitrite concentrations within each range and some overlapping between adjoining ranges, it can be seen that between $10^5$ and $10^7$ bacteria/ml, nitrite concentrations clearly rise with increased bacterial concentrations. At higher bacterial concentrations, this trend moderates. However, in terms of nitrite concentration, two main ranges can be distinguished: a lower range of $\leq 5$ ppm nitrite, which corresponds to a bacterial concentration of $10^5$ to $10^9$/ml, and an upper range of $> 5$ ppm (mostly 10 to 50 ppm) nitrite, which corresponds to $> 10^8$ bacteria/ml.

Since urinary nitrate is a prerequisite for bacterial nitrite reductase, its concentration may affect the quantitative accuracy of the correlation between bacterial concentration and nitrite production. Thus, a quantitative determination of urinary nitrate in the bacteriuric specimens was carried out. The results are presented in Fig. 3. In addition, the values of nitrate plus nitrite are given, since this combined figure provides an indication of the initial concentration of nitrate before its reduction to nitrite.

It can be seen that in urine specimens with $10^5$ to $10^8$ bacteria/ml the frequency distribution of nitrate values appears to be almost identical to that of nitrate and nitrite values (Fig. 3a, b). Indeed, in these specimens, only a small part of the urinary nitrate was reduced, as expressed by the production of 0-2 to 5 ppm of nitrites (Fig. 2). In urine specimens with higher bacterial concentrations more nitrate is reduced to nitrite (Fig. 3c-f). This trend is particularly evident for samples with a bacterial concentration of $\geq 10^7$/ml, in which the nitrate is largely or even completely consumed in most cases.

**Fig. 2 Relation between nitrite concentration and the size of the bacterial population in randomly collected bacteriuric specimens.**

![Graph showing relation between nitrite concentration and bacterial population size.](image)

**Fig. 3 Nitrate and nitrate + nitrite concentrations in randomly collected bacteriuric urine specimens. Cross-hatching = $<$ 0.1 ppm; dots = 0.1-5 ppm; vertical stripes = 5-10 ppm; diagonal stripes = 10-20 ppm. (Bacterial concentration is given only in orders of magnitude.)**

**Discussion**

A simple, rapid but reliable method of testing urine specimens for bacteriuria would be of great value in case finding and follow up of UTI. The nitrite-based tests would be suitable for fulfilling this demand. However, according to several authors, the currently available nitrite tests are not sufficiently reliable. In the light of these considerations, we evaluated a new sensitive nitrite test, the SRN test, for its suitability as a reliable diagnostic tool for the detection of bacteriuria.

According to many reports, the nature of the commonly used nitrite tests imposes some limitations on their use. A prerequisite, recommended for most of the tests, is the use of first-voided morning urine, which allows a long period for nitrite to accumulate in the bladder. Moreover, three consecutive first-morning urine samples are preferred by...
some authors, as a means of improving the sensitivity of the tests. In addition, for the available nitrite tests, a high concentration of nitrate-reducing bacteria ($\geq 10^6$ bacteria/ml) is generally necessary, as is an adequate concentration of urinary nitrate. Finally, the sensitivity of these tests may be affected by the presence of interfering factors, such as phenazopyridine, blood, ascorbic acid, urobi Ninogen, or bilirubin.

Our results demonstrate that none of these factors can limit the use of the SRN test as a reliable technique for the detection of bacteriuria. As is shown by the results of our clinical study (Table 1), first-morning urine samples are not a prerequisite for the SRN test. A sensitivity of $90\%$ was obtained for this test in urine samples collected randomly throughout the day, whereas the sensitivity of the Microstix test for these samples was as low as $30\%$. Low sensitivity for the Microstix as well as for other nitrite tests has also been reported for tests on urine samples about which clear data (on retention time in the bladder) were not supplied. The fact that the SRN test obviates the need for first-morning urine samples is of great practical importance in screening for bacteriuria. The necessity for long retention times of the urine in the bladder is not convenient for large populations at risk for bacteriuria, such as children, pregnant women, or patients who suffer from incontinence, or for patients visiting a general practitioner.

The higher reliability of the SRN test as compared with the Microstix test can be understood in the light of its higher sensitivity (Table 4). Furthermore, the reliability of the SRN test was not affected by various factors which do interfere with the Microstix test, such as the colour of the urine (Table 4), high urinary pH (Fig. 3), or the presence of phenazopyridine, bilirubin, blood, or ascorbic acid or a combination of these factors. At all times, the detection ability of the SRN test remained unchanged.

The current assumption that a concentration of bacteria as high as $10^6$/ml would provide the nitrite tests with adequate accuracy is not borne out by our study. We did not find that $10^6$ bacteria/ml was the minimal effective concentration for the Microstix test, since even at higher bacterial concentrations (up to $10^9$ bacteria/ml) the test “missed” some of the bacteriuric cases (Table 3). This may result from an insufficient accumulation of nitrite in these randomly-collected urine specimens or from the presence in the urine of factors which interfere with the sensitivity of the test. In contrast, the SRN test was sufficiently sensitive to reveal bacteriuria in the majority of samples with $10^5$ bacteria/ml and in all of the specimens with higher bacterial concentrations (Table 3).

The limited sensitivity of the SRN test in the bacterial specimens containing $10^5$ bacteria/ml may be understood in the light of our results in an experimental system (data to be published). In this in vitro system, E coli B was grown on Luria broth under anaerobic conditions which facilitate the activation of the bacterial nitrate reductase. Similarly, in the bladder, this bacterial system is probably activated by the low oxygen tension of the urine. In the model system, for an initial concentration of $10^5$ bacteria/ml, a minimal incubation period of 45 min was required to obtain 0-2 to 0-4 ppm of nitrite, detectable by the SRN test. For higher bacterial concentrations ($\geq 10^6$/ml), however, a much shorter incubation time is required for producing detectable concentrations of nitrite, since after 15 min of incubation the concentration of nitrite was far higher than the detection limit of the test.

Thus, it appears that for random bacteriuric specimens containing $10^5$ bacteria/ml retention in the bladder of less than 45 min might constitute a limit to the sensitivity of the SRN test. Nevertheless, for random specimens with higher bacterial concentrations the retention time in the bladder between two urinations can be much shorter without impairing the reliability of the test. It has been stressed elsewhere that for reliable results with the nitrite tests, a high concentration of nitrate in the urine is necessary. The SRN test, however, obviates this need. Urinary nitrate concentration cannot be a limiting factor for the SRN test, if it is used as a qualitative technique for detecting bacteriuria. Even the lowest nitrate concentrations encountered in our study were sufficient to produce nitrite concentrations far above the sensitivity threshold of the SRN test, namely 0-1 ppm. In only two of the 158 bacteriuric cases in our study was there no nitrite formation, possibly as a result of the complete absence of nitrate from the urine (Fig. 3a, b).

The possibility of using the SRN test as a quantitative technique was also examined. Our results showed good correlation between nitrite concentration and the size of bacterial population for the range of $10^5$ to $10^7$ bacteria/ml, although there was a measure of scattering and overlapping of the nitrite values (Fig. 2). For the higher range of bacterial concentration ($> 10^7$ bacteria/ml), the correlation between the two variants was poor. The lack of exact correlation may reflect at least two non-controllable factors in this random system: (a) varying retention times of the urine in the bladders of patients from whom the specimens were collected (b) varying urinary nitrate concentrations, which depend largely on the intake of nitrate-containing foods.

It seems that while at lower bacterial concentrations the crucial factor affecting the scattering and
overlapping phenomena is the retention time of the urine in the bladder, at higher bacterial concentrations both the retention time in the bladder and the concentration of nitrate in the urine contribute to the lack of exact correlation. Thus, for the SRN test to serve as a quantitative technique for the detection of bacteriuria, the time of retention of the urine in the bladder and the urinary nitrate levels must be taken into consideration. However, the SRN test can be used as a highly reliable qualitative technique for the detection of nitrate-reducing bacteriuria.

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References

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