

Comparison of nitrite and dipslide methods for bacteriuria screening

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The results of screening for bacteriuria by the nitrite reaction and the dipslide method were compared. The nitrite reaction was specific in 99.8%, its sensitivity was 93.1% and its reproducibility, 100%. Specificity of the dipslide method was 94.5%.

The nitrite reaction is considered most suited for screening, owing to its specificity, satisfactory sensitivity, short time requirement and low cost. The dipslide method is not suited for screening, as apart from being expensive it yielded 5.5% false positive reactions, but proved excellent for estimating the bacterial count in nitrite positive samples.

In bacteriuria the freshly voided urine has a bacterial count of 10^5 /ml or more. In urine specimens contaminated during urination the count is mostly below 10^4 /ml [21, 29]. When the bacterial count is higher than 10^5 /ml it is called significant bacteriuria to distinguish it from contaminated urine.

Bacteriuria may occur in itself, without other clinical or laboratory signs of urinary tract infection. The significance of this isolated or monosymptomatic bacteriuria is questionable [32, 36]. Urinary tract infections are often asymptomatic and their early detection is decisive for the patient's fate. Their earliest and most persistent symptom being bacteriuria, the importance of screening has been stressed [7, 21, 27].

Bacteriuria can be demonstrated by bacteriological and chemical methods. The bacteriological methods are ba-

sed on the fact that each bacterium in the urine will form a colony on a suitable medium and the bacterial count allows to conclude to the bacteriuria being significant or the result of contamination. Since the bacteria themselves are demonstrated, the method is very sensitive but needs 24 hours culturing before the result can be read. The simplest such test is the extensively applied dipslide method. The sterile slide covered on both sides with culture medium is dipped into urine and incubated in its container for 24 hours. The quantity of urine adhering to the surface of the cultures is always identical, therefore from the density of the developing colonies the bacterial count can be estimated. Serial examinations proved that the method was reliable and equivalent with the pour plate method [3, 4, 7, 17, 26]. Sensitivity of the dipslide test remains unchanged

on incubation at room temperature, only the growth of Gram positive microorganisms is then slower [3, 4].

The chemical methods demonstrate instead of the bacteria some of their metabolic products. Since the bacteria produce different metabolic substances, the chemical methods are less sensitive. Their advantage is that they supply results within a short time, they are simple and inexpensive. The great majority of bacteria causing urinary tract infection reduces urinary nitrate to nitrite. Nitrite can promptly be demonstrated in the urine without false positive reactions, but a positive finding fails to supply information on the strain and antibiotic sensitivity of the microorganism.

With both methods, freshly voided urine is needed, since on standing the number of contaminating bacteria will be increased.

In the present study we have compared the dipslide method with the nitrite reaction.

MATERIAL AND METHODS

The examinations were performed in 6 to 14 years old children of primary schools. They were told to hold their urine for 3 to 4 hours, then to urinate into a plastic container washed with tap-water. The interval before urination could not be controlled, but when a small quantity was voided, a negative nitrite reaction was not evaluated. The urinary specimens were examined immediately.

The nitrite reaction was performed with the Griess—Hosvay reagent modified by Csokonay [10], consisting of 0.4 g alpha-naphthylamine and 0.5 g sulphanilic acid dissolved in 170 ml 20% sulphosalicylic acid.

When 4 to 5 drops of the reagent are added to about 2 ml urine, in the presence of nitrite a red colour develops within 2 minutes. In the presence of protein, a clouding as in the sulphosalicylic acid test is observed. The reagent keeps at room temperature in a dark bottle for at least 6 months.

For the dipslide method Uricult® (Orion, Helsinki) slides were used. After dipping in urine, they were incubated for 24 hours at room temperature previous to reading. Sampling and reading of results were performed in each case by the same person. When the count was 10^5 or higher the microorganism cultured on the slide was identified and its antibiotic sensitivity determined.

The examinations were performed in 3 groups.

I. The urine of 628 girls was screened by both methods.

II. Dipslide screening was performed with 257 urines. These were selected from among 6000 girls screened by the nitrite reaction. Their urine was nitrite positive, or when it was negative, the girls were suspect of bacteriuria. Suspicion of bacteriuria arose when the urine was clouded and no clearing occurred on the addition of the acid nitrite reagent, or when the child had complaints like enuresis or frequent micturition.

Group III included 51 treated girls who had a reinfection. Their urine was examined by both methods.

The children in whom the dipslide demonstrated a bacterial count of 10^5 or higher, were called back for examination and their mid-stream urine was tested for nitrite reaction, dipslide count and urinary sediment.

The children were considered bacteriuric, when

1. the nitrite reaction was positive and the dipslide revealed a count $\geq 10^5$;
2. the dipslide demonstrated a $\geq 10^5$ bacterial count at least twice and on both occasions a pure culture of the microorganism was obtained with identical antibiotic sensitivity.

In one of the children the nitrite reaction was positive on four occasions; and in all the four instances a pure culture of *Staphylococcus albus* was obtained. At screening the dipslide gave a count of 5×10^3 . The child was considered bacteriuric; the low count was due to the slow growth of staphylococci at room temperature [3, 4]; visible colonies failed to develop from each microorganism.

RESULTS

Group I. Among the unselected 628 girls screened, the urine of 623 was nitrite negative and of 5 nitrite positive. The dipslide method gave a bacterial count of $\geq 10^5$ in 39 cases and one of $\leq 5 \times 10^4$ in 589 cases (Table I).

TABLE I

| Dipslide | Nitrite | | | Total |
|----------------------|----------|----------|----------|-------|
| | positive | negative | | |
| | | No. | per cent | |
| $\geq 5 \times 10^5$ | 5 | 17 | 2.7 | 22 |
| 10^5 | — | 17 | 2.7 | 17 |
| 5×10^4 | — | 28 | 4.5 | 28 |
| 10^4 | — | 44 | 7.1 | 44 |
| $\leq 5 \times 10^3$ | — | 396 | 63.6 | 396 |
| Sterile | — | 121 | 19.3 | 121 |
| Total | 5 | 623 | 100.0 | 628 |
| Bacteriuric | 5 | — | — | 5 |

Repeated examination verified bacteriuria in only 5 among the 39 girls; the others were false positive findings. The urine of the 5 bacteriuric girls was nitrite positive.

Group II. Among the 257 selected girls a positive nitrite reaction was

obtained in 77 cases. The dipslide demonstrated a count of $\leq 10^5$ in 90 cases (Table II).

TABLE II

| Dipslide | Nitrite | | Total |
|----------------------|----------|----------|-------|
| | positive | negative | |
| $\geq 5 \times 10^5$ | 75 | 9 | 84 |
| 10^5 | — | 6 | 6 |
| 5×10^4 — | — | 12 | 12 |
| 10^4 | — | 8 | 8 |
| $\leq 5 \times 10^3$ | 1 | 100 | 101 |
| Sterile | 1 | 45 | 46 |
| Total | 77 | 180 | 257 |
| Bacteriuric | 76 | 6 | 82 |

Among the 77 children voiding nitrite positive urines 76 suffered from bacteriuria; among these a bacterial count of $\geq 10^5$ was found in 75. The dipslide of the girl with *Staphylococcus albus* bacteriuria was negative (5×10^3). In one girl a false positive nitrite reaction was observed.

Group III. In the 51 girls with reinfection the count was $\geq 10^5$ in each specimen, the nitrite reaction was positive in 50 cases.

Specificity of the nitrite reaction. In the three groups 132 positive nitrite reactions were observed: among these one was false positive. Thus among 804 non-bacteriuric cases only one was false positive, thus the specificity was 99.8%.

Sensitivity of the nitrite reaction. Routine screening was performed by both methods in 628 girls. All the 5 bacteriuric girls voided a nitrite posi-

tive urine, but the low number of positive cases does not permit to evaluate the sensitivity.

The summarized data of Groups I and II are already sufficient to determine the sensitivity. Among the 87 bacteriuric children, 81 had a nitrite positive urine, thus the sensitivity of the reaction in this material was 93.1%. This sensitivity would only be real if among the 6000 screened girls all those with bacteriuria would have been selected. The majority of bacteriuric urines is slightly clouded, thus the expectation may be justified that further examination of clouded urines would allow to detect almost every bacteriuric child. The 628 routine dipslide examinations also supported this high sensitivity, the number of cases, however, was too low for reliable evaluation.

Reproducibility of the nitrite reaction. Among the 82 girls nitrite positive at screening, 68 were suffering from persistent bacteriuria. One reaction was false positive, 13 girls had transitory bacteriuria. (It has been shown that bacteriuria disappears spontaneously in 15 to 30% of cases [6, 33, 38]). Repeated dipslide examination of the latter was negative. In our material the reproducibility of the reaction for persistent bacteriuria was 100%. The 6 girls included in Group II, who had false negative nitrite reactions, had a nitrite positive first morning urine at reexamination.

Specificity of the dipslide method. In the course of the routine examination of Group I, among the 623

non-bacteriuric girls 34 false positive dipslide results were obtained. The specificity of the method in this material was 94.5%. The incidence of false positivity was 5.5%.

Sensitivity of the dipslide method. According to data in the literature the method's sensitivity is satisfactory, corresponding to that of other bacteriological methods [3, 4, 7, 17, 26, 30, 31, 37].

DISCUSSION

Requirements of a satisfactory screening method for bacteriuria are an adequate sensitivity; an adequate specificity; simplicity; and low cost.

According to several authors, sensitivity of the nitrite reaction is unsatisfactory, between 40 and 70% [7, 15, 20, 23, 26, 39, 40]. This conclusion was drawn from examinations where the reaction was compared to bacteriological methods. In a number of cases it was not controlled whether the persons selected by bacteriological methods had real bacteriuria on repeated examination or the culture produced a false positive result. Other authors drew conclusions from urine samples brought to the laboratory after an unknown interval.

Kass [22] described that when midstream urine was examined after careful washing, in 20% of the women with a bacterial count of $\geq 10^5$ the repeated examination was negative. According to other data, with bacteriological methods the ratio of false positive reactions is even higher. By screening tests 1.5 to

13.5% urine specimens may be false positive, that is 1.3 to 7.5 times as much as the number of persons with real bacteriuria [1, 5, 11, 12, 13, 14, 16, 21, 24, 25, 32, 34, 36, 42]. The specificity of bacteriological methods is rather uncertain; in our screening examination it amounted to 94.5%. Thus, the above results reflect the low specificity of bacteriological methods rather than the low sensitivity of the nitrite reaction.

In our examinations the sensitivity of the nitrite reaction reached 93.1%.

Alwall and Lohi [2] reported on a similarly satisfactory sensitivity of the nitrite reaction: in their adult urological patients they found a sensitivity of 95%. Among clinical patients it was 70%, but the basis of comparison was a bacteriological method, without excluding the possibility of false positive reactions. Examining the first morning urine of adult women, Czerwinski et al. [8, 9] found a sensitivity of 92 to 94%, Fritz [14] 89%, Wallmark et al. [41] 84%, at 5 hours after voiding. According to Randolph and Morris [35], among female children the sensitivity of the nitrite reaction in the first morning urine is 92.8%. The two false negative girls had megacystis and the false negative reaction was due to urine retention.

When the interval after previous micturition is short, sensitivity of the reaction in adults is 25 to 50% [2, 8, 9]. The nitrite reaction is of high sensitivity only in those cases where the examination is carried out following an urination interval of 3

to 4 hours. After an appropriate interval between micturitions, all our 6 false negative patients voided nitrite positive urines.

The specificity of the nitrite reaction is satisfactory; according to the literature it exceeds 99% [2, 8, 15, 39, 41]; in our material it was 99.8%. The specificity of the dipslide method, similarly as that of other bacteriological methods depends on urine sampling. When many subjects are screened, due washing will considerably prolong the examination, but without washing there will be a number of aspecific reactions.

Both the nitrite and the dipslide tests are simple to carry out. The nitrite test supplies immediate results; the child can be ordered back promptly for reexamination. The dipslide result, on the other hand, takes 24 hours before it can be read and requires more manipulation and administration.

Finally, against the relatively high cost of the dipslide test the cost of the nitrite reaction is negligible.

Thus, for screening for bacteriuria we recommend the nitrite reaction carried out in urine voided at least 3 to 4 hours after the last micturition. The most appropriate is the first morning urine. In positive cases the bacterial count should be determined by the dipslide method. The cultured organism is then identified and its antibiotic sensitivity is determined. The positive children should report for reexamination after 3 days. When the control result is again positive, treatment should be started.

Suspect for bacteriuria are the turbid or clouded urines which do not clear up on the addition of the nitrite reagent. Large floating parts indicate the presence of vaginal exudate, whereas in the case of bacteriuria the urine is finely clouded. When such urines yield a bacterial count $\geq 10^5$, a control examination should be carried out.

Owing to its high cost and low specificity, with the 5.5% incidence of false positive results, the dipslide method is not suited for screening examinations, in agreement with the opinion of Manners et al. [28]. On the other hand, the method is excellently suited for estimating the bacterial count in nitrite positive urines suspect of significant bacteriuria.

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