Modification of the Rapid Fermentation Test for
*Neisseria gonorrhoeae*

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A modification of the rapid sugar fermentation procedure has been developed because of difficulties encountered in reproducing the reported results. Changes in the method have made the test more sensitive and reproducible.

Kellogg and Turner (1) published a rapid sugar fermentation test for the confirmation of *Neisseria gonorrhoeae*. This rapid method was an improvement over previous methods which required growth of the organism to demonstrate fermentation. Some strains of *N. gonorrhoeae* will not ferment glucose in agar-base techniques because growth is inadequate or absent; the rapid technique requires no growth.

Several laboratories used the published procedure with many clinical strains of *N. gonorrhoeae* and were unable to obtain results comparable to those reported by Kellogg and Turner. This report presents a modification of the original procedure, which is an improvement of the rapid method for determining carbohydrate degradation by *N. gonorrhoeae*.

**MATERIALS AND METHODS**

The same materials reported by Kellogg and Turner (1) were used in this procedure. The buffer-salt solution consisted of: K$_2$HPO$_4$, 0.04 g; KH$_2$PO$_4$, 0.01 g; KCl, 0.8 g; phenol red, 0.2 ml of a 1% aqueous solution; and distilled water, 100 ml. It was prepared and stored at 5 C in a sterile, screw-cap glass bottle. The amounts required in the test procedure were dispensed into glass test tubes as needed. Concentrated solutions of glucose, maltose, lactose, sucrose, and fructose (20%) were prepared in distilled water and frozen in 5- or 10-ml amounts until needed. Once thawed, these carbohydrates were stored at 5 C. If sterility of the carbohydrates is desired, the solutions may be sterilized by membrane filtration.

Changes in the volumes of buffer-salt solutions used in the testing procedure are listed in Table 1. Glass tubes (12 by 75 mm) were used instead of plastic tubes because they were less expensive and reusable and gave satisfactory results.

One cell suspension tube and three fermentation tubes were labeled for each specimen of suspected *N. gonorrhoeae*. The cell suspension tube contained 0.3 ml of the buffer-salt solution and two loopfuls (from a 3-mm diameter loop) of a pure 24-h culture from an agar plate. A homogenous suspension was obtained by mixing the organisms with a Pasteur capillary pipette. The fermentation tubes (the tubes which are incubated) contained 0.1 ml of the buffer-salt solution. One drop (0.04 ml) of the appropriate carbohydrate (glucose, maltose, and fructose) was added to its labeled fermentation tube with a Pasteur capillary pipette. One drop (0.04 ml) from the cell suspension tube was added to each fermentation tube, which was then shaken gently by hand to mix the organisms with the carbohydrate. The fermentation tubes were incubated in a 37 C water bath. Readings could be taken after incubation times ranging from 15 min to several hours, depending on the strain of *N. gonorrhoeae* and how fast it reacted. Overnight readings were not made because of possible contamination.

**RESULTS AND DISCUSSION**

This modified method was developed primarily to improve the rapid technique of Kellogg and Turner. It gave results comparable with those reported for the original method—a positive fermentation within a 4-h period as shown

| Table 1. Suggested changes for the modified method |
|---------------------------------------------------|
| Determination | Kellogg and Turner method | Modified method |
|----------------|---------------------------|-----------------|
| Cell suspension tube | 0.25 ml of BSS* | 0.3 ml of BSS |
| Fermentation tube | 0.5 ml of BSS | 0.1 ml of BSS |
| Loopfuls of organisms | one 2-mm loopful | two 3-mm loopfuls |

* BSS, Buffer-salt solution.
in tests with 50 strains of *N. gonorrhoeae* (Table 2). When the Kellogg and Turner procedure was used exactly as published, many of the specimens never reacted, and the ones that did react usually did so after 4 h.

All specimens tested were confirmed by cystine Trypticase or Mueller-Hinton agar-base techniques. A different volume of buffer-salt solution (0.3 ml) was used for the cell suspension tube because this amount allowed one drop for five different carbohydrates, plus a reserve. Another modification was that a greater number of organisms was used (two 3-mm loopfuls) in the cell suspension tube. Results were better when a heavier inoculum than the one suggested by Kellogg and Turner was used. It is better to have too many rather than too few organisms in the cell suspension tube. An increased number of cells does not seem to greatly affect the glucose or the maltose reaction (Fig. 1).

The volume of buffer-salt solution in the fermentation tube was reduced from 0.5 to 0.1 ml. This allowed the increased inoculum to react with a higher concentration of carbohydrate. This amount was determined by studies of carbohydrate degradation by *N. gonorrhoeae*.

If the amount of glucose was increased, the gonococci seemed to react faster; however, at the same time, an increase in the amount of maltose may begin to give a positive reaction (Fig. 2). This may be due to impure commercial maltose.

The ratio of one drop of carbohydrate plus one drop of inoculum from the cell suspension tube added to 0.1 ml of buffer-salt solution seemed to give the best results. Most strains of *N. gonorrhoeae* react in glucose by changing the phenol red indicator from red to yellow. Changes of reddish-orange to orange have been observed with maltose shortly after incubation, but later the color turned back to reddish-orange or red. A few "weak" strains of gonococci will give a gold or golden-yellow color with glucose, whereas with maltose the color usually remains red.

Other species of *Neisseria* were tested to confirm the carbohydrate reactions. All reactions listed in Table 3 occurred within 2 h with the modified method as opposed to over 4 h with Kellogg and Turner's method.

An oxidase-positive culture of gram-negative diplococci that grows poorly should be subcultured to more than one plate to insure enough

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**Table 2. Length of time (hours) required to obtain positive results by the Kellogg and Turner method and by the modified method**

| Strain              | Kellogg and Turner method | Modified method |
|---------------------|---------------------------|-----------------|
|                     | > 4 h | Negative | < 1 h | 1 h | 2 h | 3 h | 4 h | > 4 h |
| *N. gonorrhoeae*    |       |          |       |     |     |     |     |       |
| Clinical strains    | 20    | 20       |       |     |     |     |     |       |
| Laboratory strains  | 3     | 7        |       |     |     |     |     |       |
| *N. meningitidis*   |       |          |       |     |     |     |     |       |
| Clinical strains    | 2     | 3        |       |     |     |     |     |       |
| Laboratory strains  | 5     |          |       |     |     |     |     |       |

*A* A positive result for *N. meningitidis* consisted of both glucose and maltose reactions.

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**Table 3. Other Neisseria species tested (laboratory strains)**

| Organism       | No. tested | Carbohydrate reactions |
|----------------|------------|------------------------|
|                |            | Glucose | Maltose | Lactose | Sucrose | Fructose |
| *N. catarrhalis* | 3          | -       | -       | -       | -       | -       |
| *N. flavescens*  | 3          | -       | -       | -       | -       | -       |
| *N. sicca*       | 3          | +       | -       | -       | +       | +       |
| *N. subflava*     | 3          | +       | +       | -       | -       | -       |
| *N. flav*a       | 3          | +       | +       | -       | -       | +       |
| *N. perflava*     | 3          | +       | +       | -       | +       | +       |
| *N. lactamica*    | 3          | +       | +       | -       | -       | -       |

*Glucose reactions for *N. subflava*, *N. flav*a, and *N. perflava* are sometimes weak or negative.
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Strain #2686 N. gonorrhoeae

- Glucose
- Maltose

**Fig. 1. Increase in inoculum** (in 0.1 ml of BSS). Growth for fermentation inoculations. Sometimes blood agar or GC Base (Difco) medium may be helpful in obtaining better growth. It is difficult to obtain a reproducible loopful of organisms when the colonies are too viscous or watery. The bacteriologist must judge whether or not the proper amount for inoculation into the cell suspension tube is obtained.

Over 100 additional clinical specimens of *N. gonorrhoeae* have been tested with the modified method. These specimens reacted positively.
TABLE 4. Additional clinical specimens tested with the modified method

| Organism          | Origin and no. of isolates | No. of isolates giving a positive result within a 4-h period |
|-------------------|----------------------------|----------------------------------------------------------|
|                   |                            | <1 h | 1 h | 2 h | 3 h | 4 h | >4 h |
| N. gonorrhoeae    | Urethra 87                 | 14   | 30  | 37  | 4   | 2   |
|                   | Cervical 44                | 11   | 13  | 18  | 2   |     |
|                   | Throat 11                  | 2    | 5   | 4   |     |     |
|                   | Blood 7                    | 3    | 3   | 3   | 1   |     |
|                   | Rectal 5                   | 2    | 2   | 2   |     |     |
|                   | Vaginal 3                  | 1    | 2   | 2   |     |     |
|                   | Knee fluid 2               | 1    | 2   | 2   |     |     |
|                   | Gastric 1                  | 1    | 1   | 1   |     |     |
|                   | Urine 1                    | 1    | 1   | 1   |     |     |
| N. meningitidis   | Urethra 1                  | 1    | 1   |     |     |     |
|                   | Blood 1                    | 1    | 1   |     |     |     |
|                   | Throat 12                  | 5    | 6   | 6   | 1   |     |
|                   | CSF* 2                     | 1    | 1   | 1   |     |     |
| N. lactamica      | Urethra 1                  | 1    | 1   |     |     |     |
| N. perflava*      | CSF 1                      |      |     |     |     | 1*  |

*CSF, cerebral spinal fluid.
*The glucose reaction for N. perflava was weak.

with glucose and negatively with maltose within a 4-h period (Table 4). Two strains of Moraxella osloensis obtained from vaginal cultures were also tested and remained negative in all carbohydrates.

This modification of the rapid fermentation technique gave more rapid and more sensitive results than the original procedure.

LITERATURE CITED

1. Kellogg, D. S., Jr., and E. M. Turner. 1973. Rapid fermentation confirmation of Neisseria gonorrhoeae. Appl. Microbiol. 25:500-52.