JOURNAL OF CLINICAL MICROBIOLOGY, July 1976, p. 19-21
Vol. 4, No. 1
Copyright © 1976 American Society for Microbiology
Printed in U.S.A.

Evaluation of Methods for the Rapid Identification of *Neisseria gonorrhoeae* in a Routine Clinical Laboratory

HELEN M. POLLOCK

Departments of Laboratory Medicine and Microbiology, University of Washington, and Harborview Medical Center, Seattle, Washington 98104

Received for publication 17 February 1976

Of 78 isolates of *Neisseria gonorrhoeae*, 21 failed to grow and produce acid in unsupplemented cystine-Trypticase agar (CTA); whereas positive reactions were obtained by using serum-supplemented CTA and fluorescent antibody (FA). An additional 290 strains of *Neisseria* were evaluated by FA and by a rapid carbohydrate degradation technique (RF). There was agreement between the two methods 92% of the time on the initial trial and 99% of the time with repeats on discrepancies. The RF and FA tests provided rapid and reliable identification of *N. gonorrhoeae*, alleviating the problems of CTA due to lack of growth and need for overnight incubation.

The rising incidence of gonorrhea and the increased necessity for following up contacts make it important to confirm suspicious isolates as rapidly as possible.

Laboratory diagnosis presently continues to depend primarily on culture and carbohydrate degradation tests, because it has been shown that culture on selective media has greater sensitivity and accuracy than the delayed fluorescent antibody (FA) method (8, 9). Unfortunately, results may take 3 days or more using these standard methods, partly because the fastidiousness of many primary isolates prevents them from growing adequately in the semisolid cystine-Trypticase agar (CTA) media which are still recommended (6). Recently, Faur et al. have described a plated medium that gave better growth and more reliable results than CTA but which still required overnight incubation (2).

In 1973, Kellogg and Turner (6) described a rapid method that depends on enzyme activity in a heavy inoculum rather than on growth, thus avoiding the problems imposed by fastidious strains. Brown subsequently described a modification of this technique which he reported gave more reliable results (1).

Difficulties with detection of acid production in CTA medium, and the delays which resulted, led us to compare this procedure with Brown's modification of the Kellogg rapid method (RF) and with a direct FA test made on isolated colonies. This comparison was followed by a prospective evaluation of a combination of the RF and FA procedures only in a routine clinical laboratory setting.

MATERIALS AND METHODS

Neisseria strains. Stock strains of *Neisseria gonorrhoeae* and *Neisseria meningitidis* were used to control medium and FA reagent performance with each test run. They had been isolated from clinical specimens, and their identities were confirmed by the Seattle-King County Public Health Laboratory. They were suspended in equal parts of fetal calf serum and Trypticase soy broth (BBL) with 0.1% yeast extract and maintained at −70°C in a Revco freezer until use.

The strains of *Neisseria* studied were routine isolates from urethral, endocervical, rectal, and pharyngeal specimens which were submitted for diagnosis of gonorrhea.

Isolation media. Isolation media were obtained from the University of Washington Hospital Media Preparation Laboratory. Chocolated 5% sheep blood agar plates were prepared with GC agar base (BBL) containing Isovitalex (BBL), and chocolated vancomycin-colistimethate-nystatin medium was prepared according to the formula of Thayer and Martin (14). Cultures were incubated in candle extinction jars at 35°C.

Carbohydrate degradation tests. Single colony isolates were subcultured to chocolated 5% sheep blood agar plates, and overnight growth from this medium was used to inoculate the tests. Unsupplemented CTA media containing glucose, maltose, sucrose, and lactose (Difco) were inoculated by seeding a heavy peptone-water suspension of the pure culture onto the surface of the medium with a capillary pipette (13). Results were read at 24 and 48 h, and tests were not considered negative until a 48-h incubation was complete.

The RF method was performed as described by Brown (1), and tubes were seeded with a dense suspension from the purified chocolated 5% sheep blood agar plates culture. Several loopfuls of the organism were emulsified in 9 drops of the phenol

red balanced salts solution, and then 1 drop was inoculated into 4 drops of each of the sugar solutions. The sugar solutions were made by mixing five parts of phenol red balanced salts solution with two parts of 20% sugar in phenol red balanced salts solution. These were portioned to test tubes in 5-ml quantities, frozen, and thawed as needed. Results of the tests were read at 2 and 4 h.

FA test. The FA tests were performed using FA-*N. gonorrhoeae* (Difco). The conjugate was used as directed in the package insert, except that *N. meningitidis* was used as the negative control instead of *Enterobacter cloacae*. The smears were read using a Zeiss RA microscope with an HBO-200 mercury-arc lamp.

Arbitration of discrepant results. The Seattle-King County Health Department served as a reference laboratory for strains showing discrepant results in the first part of the study. They used CTA medium supplemented with 1% rabbit serum for carbohydrate fermentation tests, and this yielded good growth with all isolates.

RESULTS

CTA and FA tests were compared first with 81 successive *Neisseria* isolates. Strains giving discrepant results with the two procedures were sent to the reference laboratory for identification. The results are shown in Table 1. Seventy-eight of the strains were identified as *N. gonorrhoeae* by carbohydrate degradation criteria on primary tests or in the reference laboratory. All of these were FA positive. Twenty-one (27%) strains of *N. gonorrhoeae* grew poorly or not at all in the unsupplemented CTA medium used in our laboratory and thus failed to produce acid from dextrose after a 48-h incubation. All strains were positive in the medium supplemented with rabbit serum which was used by the reference laboratory. Three strains were negative by FA and failed to produce acid from carbohydrates. They were identified as *Neisseria catarrhalis*, grew well in the unsupplemented CTA media, and produced clearly alkaline reactions in these media. Thus, in this limited series, the FA test was accurate and useful. Failure of obvious acid production from glucose in CTA without added serum appeared to reflect poor growth in the unsupplemented medium.

The RF method was first compared with FA and CTA reactions in unsupplemented media using 17 isolates. The results shown in Table 2 indicate excellent correspondence between RF and FA but indicate the same poor performance of CTA as judged by the results of the other two methods.

Based on the above results, on previous studies with the RF (1, 6) and FA methods (5, 11), and on the recommendations of Kellogg (4), it was concluded that the RF and FA both had greater sensitivity than the commonly used CTA sugars. For the following 8 months, therefore, 290 consecutive isolates of presumptive *Neisseria* were tested by these two methods. All strains showing discrepancies between the results of the two procedures were retested to attempt to resolve the differences. The findings are given in Table 3. There was an 8% discrepancy on initial testing, but only 1% failed to correspond on repeat testing. One of these strains was identified as *N. meningitidis* and two were identified as *N. gonorrhoeae*.

In the five instances in which fermentation results with *N. gonorrhoeae* showed acid production in several tubes, the initial inoculum was found to have been mixed as determined by quality control check plates. In three instances, acid production from glucose did not occur with gonococci on the first test and was found to be due to an insufficient inoculum. In the single instance in which a meningococcus gave the reaction of *N. gonorrhoeae*, the failure of the maltose reaction was also shown to be due to an insufficient inoculum.

If the mixed cultures are excluded, only 3/227 (1.3%) of the gonococcal isolates would have

TABLE 1. *Comparison of CTA, CTA supplemented with 1% rabbit serum, and FA microscopy for the identification of N. gonorrhoeae*

Strains (no.)	CTA[a]	CTA + serum[a]	FA	Identification
21	−	+	+	*N. gonorrhoeae*
57	+	NT[b]	+	*N. gonorrhoeae*
3	−	NT	−	*N. catarrhalis*

[a] Results were from 48-h readings. +, Acid production; −, no acid production.
[b] NT, Not tested.

TABLE 2. *Comparison of FA results with two carbohydrate degradation methods*[a]

Isolates (no.)	CTA (48 h)	RF (4 h)	FA
11	+	+	+
6	−	+	+

[a] +, Acid production; −, no acid production.

TABLE 3. *Number of trials necessary to achieve agreement between FA and rapid carbohydrate degradation tests with respect to the separation of N. gonorrhoeae from other species of Neisseria*

Trial	Both tests positive (no. [%])	Both tests negative (no. [%])	No. of isolates with discrepancies (no. [%])
First test	212 (73)	55 (19)	23 (8)
Second test on 23 isolates with discrepancies	15 (5)	5 (2)	3 (1)
Total	227 (78)	60 (21)	

been missed in the initial RF tests, and 1/60 (1.6%) of nongonococcal isolates would have been identified as gonococci. Three (1.3%) strains of *N. gonorrhoeae* gave equivocal results, and five (2.2%) strains gave negative results with FA on first testing. Three (5%) strains of nongonococcal *Neisseria* gave positive FAs in the first series of tests. Since the controls were adequate in these runs, the results appear to reflect the inherent variability in the FA method, which is thus greater than the RF procedure. Therefore, the three strains giving continuously discrepant results were identified on the basis of their carbohydrate utilization patterns. These results were confirmed by the reference laboratory.

DISCUSSION

Inadequate growth in CTA medium without supplementation caused delays in the confirmation of results due to the need of prolonged incubation and repeated testing. Enrichment fluids such as heated bovine albumin (10), placenta broth (3, 12), ascitic fluid (3), and yeast autolysate, and hemolyzed erythrocytes (2) have been added to improve the growth of primary isolates. It has been suggested that inadequate growth in many carbohydrate media may be the result of strains having various nutritional requirements (6, 12). Knapp and Holmes reported that 39% of the isolates from nondisseminated gonococcal infections in Seattle had requirements for arginine, hypoxanthine, and uracil (7). This was a greater number of this auxotrophic type than had been reported from strains studied in other geographical locations. The prevalence of this fastidious auxotrophic type in the community may have contributed to the large number of isolates which gave inadequate results in this medium.

In the 290 tests performed, 3.1% of the RF sugar reactions were incorrect on the first trial either due to insufficient or mixed inocula. Of the FA tests, 3.7% were incorrect and 1.5% equivocal on the first trial. Technical variation and necessary repeats on the equivocal reactions were more common with FA than with the RF. These tests showed great agreement in the identification of *N. gonorrhoeae*. Of the strains tested, 92% were identified on the first trial with total agreement between the two methods, whereas repeat testing on discrepant results raised the agreement to 99%. Both of these tests may be read on the same day they are set up and can be usefully run with repeat studies being made on any discrepancies.

LITERATURE CITED

1. **Brown, W. J.** 1974. Modification of the rapid fermentation test for *Neisseria gonorrhoeae*. Appl. Microbiol. 27:1027-1030.
2. **Faur, Y. C., M. H. Weisburd, and M. E. Wilson.** 1975. Carbohydrate fermentation plate medium for confirmation of *Neisseria* species. J. Clin. Microbiol. 1:294-297.
3. **Juhlin, O.** 1963. A new fermentation medium for *N. gonorrhoeae*, HAP-medium. Influence of different constituents on growth and indicator colour. Acta Pathol. Microbiol. Scand. 58:51-71.
4. **Kellogg, D. S.** 1974. *Neisseria gonorrhoeae* (Gonococcus), p. 124-129. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology. Washington, D.C.
5. **Kellogg, D. S., Jr., and W. E. Deacon.** 1964. A new rapid immunofluorescent staining technique for identification of *Treponema pallidum* and *Neisseria gonorrhoeae*. Proc. Soc. Exp. Biol. Med. 115:963-965.
6. **Kellogg, D. S., Jr., and E. M. Turner.** 1973. Rapid fermentation confirmation of *Neisseria gonorrhoeae*. Appl. Microbiol. 25:550-552.
7. **Knapp, J. S., and K. K. Holmes.** 1975. Disseminated gonococcal infections caused by *Neisseria gonorrhoeae* with unique nutritional requirements. J. Infect. Dis. 132:204-208.
8. **Lind, I.** 1969. Combined use of fluorescent antibody technique and culture on selective medium for the identification of *N. gonorrhoeae*. Acta Pathol. Microbiol. Scand. 76:279-287.
9. **Martin, J. E., W. L. Peacock, and J. D. Thayer.** 1965. Further studies with a selective medium for cultivating *Neisseria gonorrhoeae*. Br. J. Vener. Dis. 41:199-201.
10. **Mullaney, P. J.** 1956. A simple fermentation medium for *Neisseria gonorrhoeae*. J. Pathol. Bacteriol. 71:516-517.
11. **Peacock, W. L., Jr., B. G. Welch, J. E. Martin, Jr., and J. D. Thayer.** 1968. Fluorescent antibody technique for identification of presumptively positive gonococcal cultures. Public Health Rep. 83:337-339.
12. **Reyn, A., and M. W. Bentzon.** 1961. Possible effects of antibiotic treatment on the sensitivity and growth requirements of *Neisseria gonorrhoeae*. Bull. W.H.O. 24:333-342.
13. **Thayer, J. D.** 1970. Gonorrhea. *In* H. L. Bodily, E. L. Updyke, and J. D. Mason (ed.), Diagnostic procedures for bacterial, mycotic and parasitic infections, 5th ed. American Public Health Association, Inc. New York.
14. **Thayer, J. D., and J. E. Martin.** 1966. Improved medium selective for cultivation of *N. gonorrhoeae* and *N. meningitidis*. Public Health Rep. 81:559-562.