JOURNAL OF CLINICAL MICROBIOLOGY, July 1980, p. 15-17
0095-1137/80/07-0015/03$02.00/0

Vol. 12, No. 1

Evaluation of the Phadebact Gonococcus Test for Confirmation of *Neisseria gonorrhoeae*

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Strains of *Neisseria gonorrhoeae* grown on Thayer-Martin medium and media with and without serum were examined by the Phadebact Gonococcus Test. By using the direct method, in which colonies from growth media were mixed directly with the reagents on a microscope slide, Thayer-Martin medium, contrary to the manufacturer's claim, was not found to be the medium giving best results, and presence or absence of serum in the media did not affect the results. A large number of inconclusive results were obtained. A modification of the alternative procedure, which uses a heated suspension of the gonococcal cells as the antigen, is described. Of the 432 strains of *N. gonorrhoeae* examined by this method, 427 were accurately identified. Five strains, however, gave false-negative results. None of the 80 strains of *N. meningitidis* gave positive results. Nine of nineteen strains of *N. lactamica*, however, gave clear-cut positive results. Use of the *o*-nitrophenyl-β-D-galactopyranoside test in conjunction with the Phadebact Gonococcus Test, particularly on isolates from the pharynx, is recommended.

In the identification of *Neisseria gonorrhoeae*, isolation of typical colonies of gram-negative diplococci which are oxidase positive constitutes a presumptive identification requiring confirmation (5). Of the methods available for this purpose, the carbohydrate utilization tests and the direct fluorescent-antibody technique are the most commonly used methods. Problems with and limitations of these procedures are well known (2, 5, 8, 10).

A rapid slide agglutination test, utilizing the principle of co-agglutination, for serological identification of *N. gonorrhoeae* was described by Danielson and Kronvall (3). A commercial test kit based on the coagglutination technique was developed as the Phadebact Gonococcus Test (Pharmacia Diagnostics AB, Uppsala, Sweden). The prototype and the kit have been previously evaluated, and some of their shortcomings have been elucidated (2, 3). The kits now available incorporate improved reagents.

The purpose of this study was (i) to evaluate the Phadebact Gonococcus Test, particularly with respect to use of the Thayer-Martin (TM) medium and other growth media since TM medium is recommended by the manufacturer as the medium giving the best results; (ii) to examine effects of serum-containing media on results of coagglutination. Direct examination is not recommended by the manufacturer for growth on serum-containing media, and there have been conflicting reports by previous investigators concerning the effect of serum-containing media on the results of coagglutination (1, 6).

MATERIALS AND METHODS

***Neisseria* strains.** A total of 472 strains of *N. gonorrhoeae*, 80 of *N. meningitidis*, 7 of *Branhamella catarrhalis*, 19 of *N. lactamica*, and 2 strains each of *N. subflava* and *N. sicca* were examined. These organisms were 24- to 48-h direct cultures or 24-h subcultures of isolates from urogenital, rectal, and pharyngeal specimens received at the Provincial Laboratories of Public Health in Calgary and Edmonton, Canada.

Strains of *N. gonorrhoeae* examined were identified by the direct fluorescent-antibody technique (Difco Laboratories, Detroit, Mich.) and carbohydrate utilization with medium described by Flynn and Waitkins (4). Other *Neisseria* species were identified by carbohydrate utilization reactions and additional bacteriological procedures as necessary (2).

Growth media. Media on which the strains were grown for testing were TM medium (9) with vancomycin (3 μg/ml), colistin (7 μg/ml), trimethoprim (3 μg/ml), and amphotericin B (5 μg/ml); Edmonton Provincial Laboratory medium prepared from Tinsdale base (Difco) and a supplement providing a final concentration in the medium of 0.71% osmotically lysed sheep erythrocytes, 8.7% bovine serum, 1.0% IsoVitaleX (BBL Microbiology Systems), and 0.39% disodium phosphate, with antimicrobial agents as indicated for the TM medium except that polymyxin B in the concentration of 2.4 μg/ml replaced colistin; and Imferon agar (7).

Phadebact Gonococcus Test. The Phadebact Gonococcus Test kit contains a vial of gonococcal reagent composed of rabbit anti-gonococcal immuno-

globulin G antibodies coupled to the protein A of heat-killed staphylococci, and a vial of control reagent composed of immunoglobulin G from nonimmunized rabbits coupled to the protein A of heat-killed staphylococci. The two procedures recommended for carrying out the test are as follows. (i) In the direct procedure, colonies being tested are emulsified in a drop each of the test and control reagents on two separate parts of a microscope slide, and coagglutination is observed after rocking the slide for up to 2 min and interpreted according to the chart in the package insert. (ii) In the alternative procedure, recommended for strains giving inconclusive results in the direct procedure, colonies being tested are suspended in 0.5 ml of distilled water and heated to 80 to 100°C for 20 min, and a drop of this suspension is used to carry out the test. On examination, all 20 strains which gave inconclusive results by the direct procedure developed a strong reaction by the alternative procedure between 4 and 9 min. A heavier suspension produced agglutination more rapidly than lighter suspension. A difference in speed and strength of reactions was not observed by reducing the boiling time from 20 to 5 min. Consequently, the following improved procedure was adapted for this evaluation.

A dense suspension of the organism was prepared in 0.5 ml of sterile distilled water in a small test tube, homogenized by use of a Vortex mixer, held for 5 min in a boiling-water bath, and then centrifuged at 2,400 rpm for 10 min. With a Pasteur pipette, all but three drops of the supernatant were discarded. The bacterial cells were resuspended, and this suspension constituted the antigen for the improved procedure. With Pasteur pipettes delivering 40 drops to 1 ml, a drop of each of the test and control reagents was delivered to two separate areas of a microscope slide. One drop of the cell suspension was added to each drop, and the slide was rocked gently and examined for coagglutination. Coagglutination in the test reagent and no reaction in the control reagent were considered positive criteria for *N. gonorrhoeae*. Coagglutination in the test and control reagents or no reaction in either was considered a negative criterion for *N. gonorrhoeae*.

RESULTS

Forty strains of *N. gonorrhoeae* grown in each of the three media were examined by the direct procedure. Results are presented in Table 1. Growth on TM medium was generally found to be adherent and sticky and could not be easily emulsified into the drops of reagents. Growth from Edmonton Provincial Laboratory medium and Imferon agar emulsified better, and Imferon was the better of the two in this respect. As a result of poor emulsification, clumps and strands were present in both the test and the control reagent in most instances, and the coagglutination lattice tended to adhere to these, making differentiation between the test and the control reactions difficult. It was observed that a certain amount of experience with the test was necessary to read and interpret the reaction accurately.

In the improved procedure, 432 strains of *N. gonorrhoeae* grown on three different media were tested. After boiling, the suspension which had been blended on a Vortex mixer provided a uniform suspension free of clumps and strands. Consequently, the appearance of coagglutination in the test reagent was well contrasted with the negative results of the control. The speed and strength of the reaction for the positive tests were increased, with coagglutination occurring within 30 to 60 s with most strains. Few strains required an additional 60 s. Results of testing of different *Neisseria* species and *B. catarrhalis* are presented in Table 2. No difference in reactions was observed in tests with growth from TM medium, Edmonton Provincial Laboratory medium, and Imferon agar. All the strains of *N. gonorrhoeae* that gave negative results were correctly identified by fluorescent antibody. One strain of *N. meningitidis* gave a weak coagglutination in both the test and control reagents. This reaction was interpreted as a negative result for *N. gonorrhoeae*.

DISCUSSION

In the present evaluation of the test performed by the direct procedure, a large number of inconclusive results were obtained, making this procedure unacceptable for routine use. TM me-

TABLE 1. *Results of testing by direct procedure for the Phadebact Gonococcus Test of strains of N. gonorrhoeae grown on media with and without serum*

Medium	No. positive (%)	No. negative (%)	No. noninterpretable (%)
Edmonton Provincial Laboratory[a]	22 (55)	1 (2.5)	17 (42.5)
TM[b]	20 (50)	2 (5)	18 (45)
Imferon agar[b]	28 (70)	10 (25)	2 (5)

[a] Serum-containing medium.
[b] Serum-free media.

TABLE 2. *Results obtained by testing different Neisseria species and Branhamella with the improved procedure of the Phadebact Gonococcus Test*

Organism	Coagglutination reactions		
	Test +, control −	Test −, control −	Test +, control +
N. gonorrhoeae	427	5	0
N. meningitidis	0	79	1
B. catarrhalis	0	0	7
N. lactamica	9	10	0
N. subflava	0	2	0
N. sicca	0	2	0

dium was found not to be the medium of choice, and the presence or absence of serum in the growth medium did not affect the results. The latter finding is at variance with that of Menck, who reported that use of serum-free growth medium improved coagglutination results (6). Our findings are similar to those of Barnham and Glynn in that the presence or absence of serum in the culture medium made little difference to the test results (1).

With the improvements made to the alternative procedure, a suspension free of clumps and strands was obtained by use of a Vortex mixer. Concentration of cells by centrifugation not only enabled the test to be performed when a relatively small amount of growth was present on media, but also increased the strength and speed of reaction. The boiling time was reduced from 20 to 5 min. The use of a smaller volume of reagents made the test more economical. Results were not affected by the medium used for culture growth. Although the majority of gonococcal strains were definitively identified, occasional strains gave negative results. Among non-gonococcal *Neisseria*, false-positive results were obtained only with *N. lactamica*. It is therefore considered necessary to use the *o*-nitrophenyl-β-D-galactopyranoside test, which is rapid and easy to perform, in conjunction with the Phadebact Gonococcus Test, particularly in examination of isolates from the pharynx.

The Phadebact Gonococcus Test performed by the method described here provides a simple, economic means for rapid identification of *N. gonorrhoeae*. It should have application both in laboratories dealing with large numbers of *N. gonorrhoeae* isolates and in those handling smaller numbers, particularly in the latter group where carbohydrate utilization tests with their inherent problems are often the only methods available for confirmation.

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