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Evaluation of the Phadebact Gonococcus Test, a Coagglutination Procedure for Confirmation of *Neisseria gonorrhoeae*

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Rapid and accurate immunological confirmation of presumptively positive gonococci could be facilitated with the Phadebact Gonococcus Test, a slide coagglutination procedure. The test was compared with carbohydrate utilization and fluorescent-antibody tests on 235 clinical isolates. With the coagglutination procedure, 97.1% of the isolates were identified as compared with 93.1% by carbohydrate utilization and 98.7% by fluorescent antibody. The Phadebact test was highly specific, showing no cross-reactions with 55 other *Neisseria* species or with 50 miscellaneous organisms occasionally found growing on selective culture media. Because of its high sensitivity and specificity, ease of performance, and ability to provide results in 2 to 3 min, this procedure provides a suitable alternative to the carbohydrate utilization and fluorescent-antibody tests for confirmation of *N. gonorrhoeae*.

Confirmatory tests such as the carbohydrate utilization (CTA) or direct fluorescent-antibody (FA) tests are performed on presumptively positive cultures when specific identification of *Neisseria gonorrhoeae* is desired or required (3). CTA and other carbohydrate confirmation methods now in widespread use have one or more of the following disadvantages: (i) isolates must be purified (otherwise erroneous patterns may develop), (ii) a heavy inoculum must be used, (iii) some isolates fail to grow or grow poorly without utilizing glucose, and (iv) isolates usually require 4 h in some procedures (2, 16) or much longer in others before reactions are complete (1, 4, 9, 21-23, 27, 28). The identification of *N. gonorrhoeae* by FA techniques has been well documented, but these techniques have some objectionable features, such as: (i) some available FA reagents are not specific and cross-react with meningococci and other bacteria, (ii) costly fluorescence equipment is needed to perform the test, and (iii) occasionally an isolate of *N. gonorrhoeae* is encountered which does not fluoresce (6, 11, 20, 26). Some of these confirmatory procedures require 1 to 3 days to provide definitive results. Laboratory confirmation would be facilitated with a rapid procedure such as those used with other bacteria (5, 8, 12, 13, 17).

Coagglutination (COA) tests for laboratory identification of several organisms are presently available from commercial sources (10, 24) and are reported to be accurate, reliable, and easy to perform (14, 25). Recently, a gonococcal COA test was described (7, 15, 18) which is based on the principle that staphylococci rich in protein A on their outer surfaces bind immunoglobulin G nonspecifically through the Fc region (18), leaving gonococcal-specific Fab sites free to react with homologous gonococcal antigen. The subsequent reaction of Fab with homologous antigen is visualized by the clumping of the staphylococci.

The purpose of this study was to compare a gonococcal COA test (Phadebact Gonococcus Test, Pharmacia Diagnostics) with the CTA and FA tests, which are the most widely used and recommended conventional methods.

MATERIALS AND METHODS

Bacterial cultures. The bacterial cultures used were (i) documented stock cultures of *N. gonorrhoeae*, *N. meningitidis*, various *Neisseria* species, and other miscellaneous organisms submitted to the Center for Disease Control for identification or confirmation of identification and (ii) cultures of fresh urethral endocervical, rectal, and pharyngeal specimens obtained from male or female patients treated at venereal disease clinics at Atlanta, Ga.

After the cultures were incubated for 24 to 48 h in candle extinction jars at 35°C, *N. gonorrhoeae* was presumptively identified by typical morphology and by growth of gram-negative, oxidase-positive diplococci on Thayer-Martin, modified Thayer-Martin, or Martin-Lewis media.

For the purpose of this study, primary cultures are defined as cultures tested after 24 to 48 h of incubation of the original specimen. Secondary isolates were those specimens transferred one or more times to selective or nonselective media before confirmatory tests were performed.

COA test. The lyophilized gonococcal and control reagents were prepared in accordance with the man-

ufacturers' instructions. They were resuspended in 1.5 ml of reconstituted phosphate-buffered saline, pH 7.4, and centrifuged at 1,500 × *g* for 10 min. The supernatant was decanted, and the reagents were resuspended in 1.5 ml of buffer, transferred to a bottle with a dropper cap, and stored at 4°C. All reagents were used within 1 month after they were prepared.

The manufacturers' directions for use of the reagents were followed. Two smears of growth from the primary or secondary culture plate were made on a glass slide with a platinum loop. Each smear contained approximately a 10-mm stroke of the loop through the growth of the organism. The smears were allowed to dry at room temperature for 1 to 2 min. One drop of control reagent was mixed well with one of the smears by means of the platinum loop or applicator stick, and one drop of specific gonococcal reagent was thoroughly mixed with the other smear. The slides were then rocked slowly (10 to 15 tilts per min at an angle of 45°) and observed for COA against a dark background with oblique transillumination. Reactions were usually complete within 1 to 2 min, but if no reaction was observed, the slide was reexaminated after 2 to 3 min of additional rocking.

The tests were performed on a small glass plate with rings like those used in most serology laboratories for performing febrile agglutination slide tests or VDRL slide tests. Results were read as 3+ (strong), 2+ (moderate), 1+ (weak), or negative COA.

FA tests. One lot of fluorescein-labeled anti-*N. gonorrhoeae* globulin prepared by the Bacterial and Fungal Products Branch, Center for Disease Control, was absorbed with *N. meningitidus* and was used as the confirming reagent. The reference technique was that of Peacock (19), with two modifications: (i) the incubation time was decreased from 30 min to 5 min, and (ii) *N. meningitidis* was used as a negative control instead of *Enterobacter cloacae*. The smears were examined for the presence of fluorescing typical organisms.

CTA tests. CTA tests were performed in tubes containing cystine Trypticase agar (BBL Microbiological Systems) and 1% carbohydrate. The identification of isolates as *N. gonorrhoeae* was confirmed on the basis of the ability to produce acid in glucose, but not in maltose, sucrose, or lactose.

RESULTS

To check the specificity of the Phadebact reagent, we tested 100 *Neisseria* sp. stock cultures and 50 stock cultures of miscellaneous organisms that occasionally grow on selective media.

Correct results were obtained on 95.3% (143/150) of the stock *Neisseria* and miscellaneous organisms combined (Tables 1 and 2). The seven cultures which were read as noninterpretable on the basis of nonspecific reaction with the control reagent were retested according to the test instructions. If a noninterpretable reaction occurred, 0.5 ml of an aqueous (H_2O) suspension of several colonies was heated to 80 to 100°C for at least 20 min. Two drops of this suspension were used for testing, one each for the test and control. Results of retests were clearly negative with all seven cultures.

Primary and secondary isolates identified as *N. gonorrhoeae* were compared by CTA, FA, and COA. The total numbers and percentages of positive results obtained with the 235 clinical isolates by the three methods are presented in Table 3. Identical results by all three confirma-

TABLE 1. *Coagglutination results with 100 stock Neisseria species*

Neisseria species	No. of strains tested	No. positive	No. negative	No. noninterpretable initially[a]
N. gonorrhoeae	45	45	0	0
N. meningitidis A	22	0	20	2
N. meningitidis B	9	0	9	0
N. meningitidis C	8	0	8	0
N. meningitidis D	1	0	1	0
N. sicca	3	0	2	1
N. flava	3	0	2	1
N. subflava	2	0	2	0
N. lactamica	7	0	7	0

[a] When heated cultures were tested by an alternative procedure, all were clearly negative.

TABLE 2. *Coagglutination results with 50 miscellaneous stock organisms*

Organisms	No. tested	No. positive	No. negative	No. non-interpretable initially
Escherichia coli	4	0	4	0
Enterobacter cloacae	4	0	4	0
Staphylococcus aureus	7	0	7	0
S. epidermidis	4	0	4	0
Candida albicans	8	0	8	0
Moraxella polymorpha	8	0	7	1
M. osloensis	5	0	4	1
Branhamella catarrhalis	10	0	9	1

[a] When heated cultures were tested by an alternative procedure, all were clearly negative.

TABLE 3. *Summary of confirmatory identification results with three different methods for identifying presumptively positive N. gonorrhoeae*[a]

Confirmation procedure			Tested	% of total
CTA	FA	COA		
+	+	+	200	85.1
+	+	−	6	2.5
+	−	+	0	0
−	+	+	14	6.0
−	−	+	2	0.9
+	−	−	1	0.4
−	+	−	0	0
−	−	−	12	5.1

[a] Agreement between methods: CTA − COA = 212 − 90.2%; CTA − FA = 220 − 93.6%; FA − COA = 227 − 96.6%.

TABLE 4. *Coagglutination results for clinical isolates of species other than N. gonorrhoeae*

Species	Source	No. of strains tested	No. of positive	No. negative	Noninterpretable initially	Total negative
N. meningitidis	Pharyngeal	9	0	5	4[a]	9
N. sicca	Cervical	1	0	0	1	1
B. catarrhalis	Rectal	2	0	2	0	2

[a] Upon testing of heated cultures by an alternative procedure, all strains were clearly negative.

tory tests were obtained with 212 isolates (200 were positive and 12 were negative). Of the remaining samples, 14 were positive by both FA and COA but negative by CTA; 1 was negative by both FA and COA but positive by CTA; 6 were positive by both CTA and FA but negative by COA; and 2 were negative by CTA and FA but positive by COA. Thus, there were 220 isolates on which CTA and FA results agreed (93.6%), 212 isolates for which CTA and COA agreed (90.2%), and 227 isolates for which FA and COA agreed (96.6%).

The 12 isolates that were negative by all three methods were identified as to species as shown in Table 4. Five of these isolates and one *N. gonorrhoeae* isolate were initially read as COA noninterpretable. After the cultures were heated and retested, these also gave valid test results.

DISCUSSION

Although CTA and FA techniques have been widely used for confirming the identification of *N. gonorrhoeae*, a reliable and more convenient method for such confirmation is needed.

The Phadebact Gonococcus Test was developed for use in confirming presumptively positive results obtained with primary isolates on selective media or with transfers (secondary isolates) from such media. Confirmatory test results were available within 2 to 3 min. Use of growth from the primary isolation plate depended upon the amount of presumptively positive growth and the number and distribution of contaminating colonies. When growth on primary isolation plates was insufficient, a secondary culture with sufficient growth was available for testing the next morning. In this study, only four cultures required transfer, because with this test a definitive result could be obtained with as few as 10 colonies.

The test as performed was not difficult to read. Approximately 60% of the isolates gave strong (3+) COA reactions, 21% yielded moderate (2+) reactions, and 12% gave weak (1+) reactions in 2 to 3 min. The strongest COA results were obtained with 18- to 24-h primary and secondary cultures. Occasionally, cultures that had been incubated for as long as 48 h were difficult to emulsify. Oxidase-treated cultures did not lose their ability to coagglutinate. Neither use of different media for growth of the isolates (Thayer-Martin, modified Thayer-Martin, Martin-Lewis, or CA) nor use of secondary instead of primary cultures affected the COA test.

Olcen (18) recently reported that 2% of the isolates tested with the Phadebact test gave noninterpretable results, i.e., COA occurred in the test and control. We found 3% of the isolates to be noninterpretable. However, noninterpretability was not a problem when the alternative boiling procedure was used.

In this study, definitive COA test results for 96.4% (212/220) of the presumptively positive gonococcal cultures were available 2 to 3 min after the primary specimen was received.

The reagents tested in our study maintained their activity for at least 1 month after rehydration when stored at 4°C as recommended by the manufacturer. This duration should be adequate for use in a clinical laboratory.

The importance of being able to confirm identification the day after the specimen is obtained, when indicated, is that confirmation could be made early enough for the results to be useful in clinical practice and for epidemiological studies.

The results of this study show that the Phadebact Gonococcus Test now offers the clinical laboratory a sensitive, specific, and easy-to-perform method which provides a suitable alternative to CTA and FA tests for confirmation of *N. gonorrhoeae*.

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LITERATURE CITED

1. **Baron, E. S., and A. K. Saz.** 1976. Effect of types of media on the production of acid from glucose by so-called glucose-negative strains of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **3**:330–333.
2. **Brown, W. J.** 1976. A comparison of three fermentation methods for the confirmation of *Neisseria gonorrhoeae*. Health Lab. Sci. **13**:54–58.
3. **Catlin, B. W.** 1973. Nutritional profiles of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Neisseria lactamica* in chemically defined media and the use of growth requirements for gonococcal typing. J. Infect.

Dis. **128**:178-194.
4. **Center for Disease Control.** 1975. Criteria and techniques for the diagnosis of gonorrhea. Center for Disease Control, Atlanta, Ga.
5. **Christensen, P., G. Kahlmeter, S. Jonsson, and G. Kronvall.** 1973. New method for the serological grouping of streptococci with specific antibodies absorbed to protein A-containing staphylococci. Infect. Immun. **7**: 881-885.
6. **Danielsson, D.** 1963. The demonstration of *Neisseria gonorrhoeae* with the aid of fluorescent antibodies. I. Immunological studies of antigonococcal sera and their fluorescein-labelled globulins, with particular regard to specificity. Acta Dermatol. Venereol. **43**:451-464.
7. **Danielsson, D., and G. Kronvall.** 1974. Slide agglutination method for the serological identification of *Neisseria gonorrhoeae* with anti-gonococcal antibodies adsorbed to protein A-containing staphylococci. Appl. Microbiol. **27**:368-374.
8. **Edwards, E. A., and R. L. Hilderbrand.** 1976. Method for identifying *Salmonella* and *Shigella* directly from the primary isolation plate by coagglutination of protein A-containing staphylococci sensitized with specific antibody. J. Clin. Microbiol. **3**:339-343.
9. **Faur, Y. C., M. H. Weisburd, and M. E. Wilson.** 1975. Carbohydrate fermentation palte medium for confirmation of *Neisseria* species. J. Clin. Microbiol. **2**:178-182.
10. **Hahn, G., and I. Nyberg.** 1976. Identification of streptococcal groups A, B, C, and G by slide co-agglutination of antibody-sensitized protein A-containing staphylococci. J. Clin. Microbiol. **4**:99-101.
11. **Juhlin, I.** 1963. A new fermentation medium for *Neisseria gonorrhoeae* HAP-Medium. Influence of different constituents on growth and indicator color. Acta Pathol. Microbiol. Scand. **58**:51-71.
12. **Juhlin, I., and S. Winblad.** 1973. Serotyping of mycobacteria by a new technique wing antibody globulin adsorbed to staphylococcal protein A. Acta Pathol. Microbiol. Scand. Sect. B **81**:179-180.
13. **Kronvall, G.** 1973. A rapid slide-agglutination method for typing pneumococci by means of specific antibody adsorbed to staphylococci protein. J. Med. Microbiol. **6**: 187-190.
14. **Leland, D. S., R. C. Lachapelle, and F. M. Wlodarski.** 1978. Method for rapid detection of group B streptococci by coagglutination. J. Clin. Microbiol. **7**:323-326.
15. **Menek, H.** 1976. Identification of *Neisseria gonorrhoeae* in cultures from tonsillo-pharyngeal specimens by means of a slide co-agglutination test (phadebact gonococcus test). Acta Pathol. Microbiol. Scand. Sect. B **84**:139-144.
16. **Morse, S. A., and L. Bartenstein.** 1976. Adaptation of the Minitek system for the rapid identification of *Neisserai gonorrhoeae*. J. Clin. Microbiol. **3**:8-13.
17. **Olcen, P., D. Danielsson, and J. Kjellander.** 1975. The use of protein A-containing staphylococcal sensitized with anti-meningococcal antibodies for grouping *Neisseria meningitidus* and demonstration of meningococcal antigen in cerebrospinal fluid. Acta Pathol. Microbiol. Scand. Sect. B **83**:387-396.
18. **Olcen, P., D. Danielsson, and J. Kjellander.** 1978. Laboratory identification of pathogenic *Neisseria* with special regard to atypical strains: an evaluation of sugar degradation, immunofluorescence and co-agglutination tests. Acta Pathol. Microbiol. Scand. Sect. B **86**:327-334.
19. **Peacock, W. L.** 1970. Method for preparing *Neisseria gonorrhoeae* fluorescent antibody conjugate. Public Health Rep. **85**:733-738.
20. **Peacock, W. L., B. G. Welch, J. E. Martin, Jr., and D. Thayer.** 1968. Fluorescent antibody technique for identification of presumptively positive gonococcal cultures. Public Health Rep. **83**:337-340.
21. **Pollock, H. M.** 1976. Evaluation of methods for the rapid identification of *Neisseria gonorrhoeae* in a routine clinical laboratory. J. Clin. Microbiol. **4**:19-21.
22. **Reddick, A.** 1975. A simple carbohydrate fermentation test for identification of the pathogenic *Neisseria*. J. Clin. Microbiol. **2**:72-73.
23. **Reyn, A., and M. W. Bentson.** 1961. Possible effects of antibiotic treatment on the sensitivity and growth requirements of *Neisseria gonorrhoeae*. Bull. W.H.O. **24**: 333-342.
24. **Rosher, R.** 1977. Laboratory evaluation of a rapid four-hour serological grouping of groups A, B, C, and G beta-streptococci by the phadebalt streptococcus test. J. Clin. Microbiol. **6**:23-26.
25. **Stoner, R. A.** 1978. Bacitracin and coagglutination for grouping of beta-hemolytic streptotocci. J. Clin. Microbiol. **7**:463-466.
26. **Tramont, E. C., J. C. Sadoff, and M. S. Artenstein.** 1974. Cross-reactivity of *Neisseria gonorrhoeae* and *Neisseria meningitidis* and the nature of antigens involved in the bactericidal reaction. J. Infect. Dis. **130**: 240-247.
27. **Wallace, R., F. Ashton, F. Charron, and B. B. Diena.** 1975. An improved sugar fermentation technique for the confirmation of *Neisseria gonorrhoeae*. Can. J. Public Health **66**:251-252.
28. **White, L. A., and D. S. Kellogg, Jr.** 1965. An improved fermentation medium for *Neisseria gonorrhoeae* and other *Neisseria*. Health Lab. Sci. **2**:238-241.