

Serologic Confirmation of *Neisseria gonorrhoeae* by Monoclonal Antibody-Based Coagglutination Procedures

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Two commercially available monoclonal antibody coagglutination tests, Phadebact Monoclonal GC OMNI Test (PMGOT; Pharmacia Diagnostics AB, Uppsala, Sweden) and GonoGen (GG; New Horizons Diagnostics, Columbia, Md.), for the confirmation of *Neisseria gonorrhoeae* were evaluated. The sensitivities of PMGOT and GG were 99.2 and 98.7% and the specificities were 91.5 and 100.0%, respectively. False-positive reactions were observed with *Neisseria lactamica* and *Neisseria meningitidis* in PMGOT. A modification of the procedure recommended by the manufacturer for PMGOT was done by substituting Todd-Hewitt broth for 0.9% saline to prepare the suspension of the test organism. This eliminated technical difficulties with the test and resulted in a sensitivity and a specificity of 99.3 and 100.0%, respectively. Advantages offered by the modified PMGOT over GG were the better predictive value of the negative test, the lower cost, the ease of reading of the test, and the lack of noninterpretable results.

Species of *Neisseria* and *Branhamella* are part of the usual bacterial flora of the human oro- and nasopharyngeal mucous membranes and are often found in genital and nongenital specimens submitted to diagnostic laboratories for the isolation of *Neisseria gonorrhoeae*. Because the diagnostic, therapeutic, social, and legal consequences of misidentification of a nongonococcal *Neisseria* isolate as *N. gonorrhoeae* can be substantial, the accurate and rapid identification of this organism is mandatory.

Carbohydrate degradation assays have long been used as a confirmatory test for *N. gonorrhoeae*. Serologic methods, such as immunofluorescent staining with labeled polyclonal antibody and coagglutination (COA) with nonviable *Staphylococcus aureus* coated with polyclonal antibody, have been used as rapid confirmatory tests, and their limitations are well documented (2, 8). In recent years, the polyclonal antibodies used in these tests have been replaced by monoclonal antibodies, which are presumed to make the tests more specific. Hence, the Phadebact Gonococcus test (Pharmacia Diagnostics AB, Uppsala, Sweden), a polyclonal antibody-based COA test, has been replaced by its manufacturer with the Phadebact Monoclonal GC OMNI Test (PMGOT; Pharmacia), which is also a COA test. In the PMGOT, a pool of murine monoclonal antibodies directed against outer membrane proteins IA and IB, corresponding to COA serogroups WI and WII/III, respectively (9, 10), is used to coat *S. aureus* cells. However, contrary to expectations, PMGOT has been shown to have various degrees of specificity and sensitivity (3-5); and to obtain accurate results, adherence to very precise test conditions, which may be difficult to achieve consistently in a diagnostic laboratory, is needed (3, 5).

Another commercially available COA test kit, GonoGen (GG; New Horizons Diagnostics, Columbia, Md.), is also based on the use of a pool of murine monoclonal antibodies prepared against purified outer membrane protein I antigens of *N. gonorrhoeae*. It has been shown to be very specific, but it fails to react with occasional gonococcal isolates (4, 7).

This study was undertaken to compare the two commercially available monoclonal antibody-based kits. We modi-

fied the recommended technique of the manufacturer for PMGOT in order to improve the specificity of the test, and in a second phase of the study we compared the modified procedure with the recommended procedure of the manufacturer.

MATERIALS AND METHODS

***Neisseria* isolates.** The study was undertaken in two phases. In the first phase, PMGOT and GG were compared by using the recommended procedures of the manufacturers for each test. In the second phase, the procedures of the manufacturers of the two tests were compared with a modified procedure for PMGOT.

(i) **Phase I.** A total of 237 isolates of *N. gonorrhoeae*, 50 of *Neisseria meningitidis*, 10 of *Neisseria lactamica*, 3 of *Neisseria cinerea*, 2 of *Branhamella catarrhalis*, 5 of *Neisseria sicca*, and 1 each of *Neisseria subflava* and *Neisseria mucosa* were examined.

(ii) **Phase II.** A total of 152 isolates of *N. gonorrhoeae*, 35 of *N. meningitidis*, 15 of *N. lactamica*, 6 of *N. cinerea*, 2 of *N. mucosa*, 1 of *N. subflava*, 1 of *B. catarrhalis*, 3 of *N. polysaccharea*, 2 of *N. sicca*, and 1 of *N. flavescens* were examined. Because of a published report (5) that several species of *Neisseria* other than *N. gonorrhoeae*, including some American Type Culture Collection (ATCC; Rockville, Md.) strains, cross-reacted in the PMGOT, the following ATCC organisms, which were tested in that study, were included: *N. subflava* ATCC 10555, *N. sicca* ATCC 29256, *N. cinerea* ATCC 14685, *N. mucosa* ATCC 19626, *N. meningitidis* ATCC 13077, *N. lactamica* ATCC 23970, *N. elongata* ATCC 25295, *N. elongata* subsp. *glycolytica* ATCC 29315, and *B. catarrhalis* ATCC 25238.

The organisms, with the exception of the ATCC organisms, were 24- or 48-h primary cultures or 24-h subcultures of isolates from urogenital, rectal, and pharyngeal specimens received at the Provincial Laboratory of Public Health, Calgary, Alberta, Canada.

Isolates of *N. gonorrhoeae* were identified by the direct fluorescent-antibody technique (Difco Laboratories, Detroit, Mich.) and by carbohydrate degradation tests as described by Flynn and Waitkins (6). Other *Neisseria* species were

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TABLE 1. Comparison of PMGOT with GG in phase I of the study

Species	No. of isolates tested	No. of isolates detected by:					
		PMGOT			GG		
		+	-	NI ^a	+	-	NI
<i>N. gonorrhoeae</i>	237	235	2	0	234	3	0
<i>N. meningitidis</i>	50	2	48	0	0	49	1
<i>N. lactamica</i>	10	4	6	0	0	10	0
<i>N. cinerea</i>	3	0	2	1	0	3	0
<i>N. mucosa</i>	1	0	1	0	0	1	0
<i>N. subflava</i>	1	0	1	0	0	1	0
<i>N. sicca</i>	5	0	5	0	0	2	3
<i>B. catarrhalis</i>	2	0	2	0	0	2	0

^a NI, Noninterpretable.

identified by carbohydrate degradation reactions and additional bacteriological procedures as necessary (8).

Growth media. For primary isolations modified Thayer-Martin medium was used as described previously (1). The same medium, but without antibiotics and antimycotic agents, was used for subcultures and for growth of the ATCC strains.

PMGOT. (i) Recommended procedure of the manufacturer. A light suspension of the organism, which was equal in density to a 0.5 McFarland standard in 0.5 ml of 0.9% saline, was boiled in a water bath for 5 min. One drop of this suspension was added to a drop of each of the test and control reagents on the disposable slide included in the kit. The drops were mixed, the slides were rocked gently, and the reaction was read within 1 min.

(ii) Modified procedure. The procedure outlined above was followed except that a heavier suspension, which was equal in density to a no. 2 McFarland standard, prepared in Todd-Hewitt broth (TH; Difco) was used instead of 0.9% saline. This procedure is referred to as TH-PMGOT.

GG. GG was used as recommended by the manufacturer. Briefly, one drop of a heavy suspension, which was equal in density to a no. 3 McFarland standard, was prepared in 0.2 ml of water and boiled for 10 min in a boiling water bath; it was then mixed with 1 drop of GG test reagent and 1 drop of control reagent on a glass slide that was provided in the kit. The slide was rocked gently, and the reactions were read within 2 min.

Interpretation of test results. In the tests described above, the presence of coagglutination in the test reagent and no reaction in the control reagent was considered a positive test result. A reaction in the test reagent, when there was a reaction in the control reagent to any extent, was considered a noninterpretable result. A reaction was considered weak when there was fine coagglutination with the background remaining dense and was considered strong when coagglutination was in large aggregates with a clear background.

RESULTS

Results of the examination of 309 isolates of *Neisseria* species in phase I of the study are presented in Table 1.

By using a saline suspension with the density of a 0.5 McFarland standard, the strength of the reactions produced in PMGOT by *N. gonorrhoeae* isolates varied from weak to strong, and the rate at which the reaction developed also varied. Some isolates gave a weak reaction at 1 min and developed a stronger one if the reaction was allowed to

TABLE 2. Comparison of 0.9% saline suspension with TH broth suspension of *Neisseria* sp. in PMGOT in phase II of the study

Species	No. of isolates tested	No. of isolates detected by PMGOT with:					
		0.9% saline			TH broth		
		+	-	NI ^a	+	-	NI
<i>N. gonorrhoeae</i>	153	152	1 ^b	0	152	1 ^b	0
<i>N. meningitidis</i>	35	2	32	1	0	35	0
<i>N. lactamica</i>	15	4	10	1	0	15	0
<i>N. cinerea</i>	6	0	5	1	0	6	0
<i>N. mucosa</i>	2	0	2	0	0	2	0
<i>N. subflava</i>	1	0	1	0	0	1	0
<i>N. sicca</i>	2	0	2	0	0	2	0
<i>N. polysaccharea</i>	3	0	3	0	0	3	0
<i>N. flavescens</i>	1	0	1	0	0	1	0
<i>B. catarrhalis</i>	1	0	1	0	0	1	0

^a NI, Noninterpretable.

^b The same isolate was positive when tested by GG.

proceed beyond that time. GG, on the other hand, produced a strong reaction within 2 min with most isolates. Because of the use of stained reagents, reactions in PMGOT were easier to read than those in GG. Two isolates of *N. gonorrhoeae* failed to react in PMGOT, and three others failed to react in GG. Two isolates of *N. meningitidis* gave a positive reaction in PMGOT when they were first isolated, but they were nonreactive when tested after subculturing. In PMGOT, one isolate of *N. cinerea* gave a strong reaction in the test reagent and a weak reaction in the control reagent. This reaction was considered noninterpretable. In GG, one isolate of *N. meningitidis* and three of *N. sicca* gave noninterpretable results.

Results of the examination of 219 isolates of *Neisseria* species by PMGOT and TH-PMGOT in phase II are presented in Table 2.

In PMGOT, with saline used to prepare bacterial suspensions, positive results were obtained with two isolates of *N. meningitidis*, which gave weak reactions, and four isolates of *N. lactamica*, which gave strong reactions. Noninterpretable results were obtained with one isolate each of *N. meningitidis* and *N. lactamica*, both of which reacted strongly in both the test and the control reagents, and one isolate of *N. cinerea*, which gave a strong reaction in the test reagent and a weak reaction in the control reagent. In TH-PMGOT, suspensions with a density greater than a no. 2 McFarland standard occasionally produced strings of organisms in both the test and control reagents, making it difficult to read the results. When such suspensions were diluted by adding TH to give a density equal to or less than a no. 2 McFarland standard and were then retested, the results were satisfactory. Strong reactions were obtained for most isolates within 1 min. One isolate of *N. gonorrhoeae* which did not react in PMGOT or TH-PMGOT was found to react positively when tested by GG.

None of the ATCC strains that were tested reacted positively in PMGOT or TH-PMGOT.

Statistical data for both phases of the study are given in Table 3.

DISCUSSION

The results of our study indicated the sensitivity of PMGOT to be about 99.0%, which is similar to that found by other investigators (3-5). Both PMGOT and GG failed to react with occasional isolates of *N. gonorrhoeae*. The iso-

TABLE 3. Sensitivities, specificities, predictive values, overall agreements, and noninterpretable results of coagglutination tests used for confirmation of *N. gonorrhoeae* in phases I and II of the study

Parameter	%			
	Test in phase I		Test in phase II	
	PMGOT ^a	GG	PMGOT ^a	TH-PMGOT ^b
Sensitivity	99.2	98.7	99.3	99.3
Specificity	91.5	100.0	90.5	100.0
Predictive value of the positive test	97.5	100.0	96.2	100.0
Predictive value of the negative test	97.0	95.8	98.3	98.5
Overall agreement	97.4	99.0	96.8	99.5
Noninterpretable results	0.3	1.3	1.4	0.0

^a To prepare bacterial suspensions, 0.9% saline was used.

^b To prepare bacterial suspensions, TH broth was used.

lates that failed to react by PMGOT were different from those that failed to react by GG. This can be expected of tests which use monoclonal antibodies directed to specific epitopes which may not be present in all strains of *N. gonorrhoeae* in different geographical areas.

The specificity of PMGOT in our study was 91.5%. This value is at variance with that observed by Carlson et al. (3) (100.0%) and Evins et al. (5) (60.0%), but it is similar to that observed by Dillon et al. (4) (91.2%). In our study, false-positive reactions were observed consistently with *N. lactamica* and were observed with *N. meningitidis* only on primary cultures. Dillon et al. (4) have reported false-positive reactions with *N. cinerea* and *B. catarrhalis*, as well as with *N. lactamica* and *N. meningitidis*, although in their study isolates of nongonococcal *Neisseria* species other than *N. lactamica* gave reactions in both test and control reagents. We considered such reactions to be noninterpretable. Evins et al. (5) also observed false-positive reactions with multiple nongonococcal *Neisseria* species, including several ATCC strains. However, we could not reproduce their results with the ATCC strains, all of which failed to react in PMGOT or TH-PMGOT.

In our study and in those of Lawton and Battaglioli (7) and Evins et al. (5), the specificity of GG was somewhat higher (100.0%) than that reported by Dillon et al. (4) (99.1%), possibly because the latter included nongonococcal *Neisseria* species, which gave noninterpretable reactions as positive reactions in their tests. Since the control reagent is made up of *S. aureus* coated with normal rabbit antibodies, coagglutination in the test reagent, when there is a reaction in the control reagent, indicates that a nonspecific reaction took place and that the identity of the isolate needs to be confirmed by another method.

While reactions with equal amounts of coagglutination in both the test and the control reagents may be easier to recognize as noninterpretable, occasional isolates may give a strong reaction in the test reagent and a weak reaction in the control reagent. While this type of reaction, which we obtained with a *N. cinerea* isolate, can be considered noninterpretable, it has the potential of being confused as a positive reaction by less experienced laboratory technologists.

To achieve a high level of specificity in PMGOT, a very precise technique, which is cumbersome to attain routinely, is needed. The density of the suspension must be equal to a

0.5 McFarland standard, and the saline for suspension preparation must be at pH 7.4 (3, 5). We found that although all *N. gonorrhoeae* isolates tested gave a strong and rapid reaction when a suspension with a density of a no. 2 McFarland standard, as in TH-PMGOT, was used, the intensity and speed of the reaction when the test was performed as recommended by the manufacturer were variable, and many strains developed only weak reactions when they were read at 1 min.

It is possible that a density of less than a 0.5 McFarland standard could give false-negative test results for such isolates. According to the product insert provided by the manufacturer, use of a suspension with a density higher than a 0.5 McFarland standard may result in a false-positive reaction. Since preparation of a suspension with a density exactly equal to a 0.5 McFarland standard is a subjective process and may vary among technologists, such variations could easily lead to both false-positive and false-negative results. Preparation of saline with a pH of 7.4, which is not buffered, is difficult and cumbersome, and the preparation needs to be fresh to avoid a reduction in pH through exposure to atmospheric CO₂ while in use.

GG has none of these disadvantages and is simple to use. No particular care is required to prepare the test suspension, since a heavy suspension equal to a no. 3 McFarland standard is required and the reactions are consistently strong and well developed within 2 min.

False-positive reactions that are observed by PMGOT with occasional isolates of *N. lactamica* and *N. meningitidis* were eliminated by the use of TH instead of 0.9% saline in the test. This resulted in a specificity of 100%, which was comparable to that of GG (100%). TH-PMGOT was also as sensitive (99.3%) as GG (98.7%), but as with the other serologic procedures, it failed to react with occasional *N. gonorrhoeae* isolates. Also, the predictive value of the negative test of TH-PMGOT (98.5%) was better than that of GG (95.8%), but it was similar to that of PMGOT (98.3%) in phase II of the study. The density of the organism suspension to be tested needed to be at or below a no. 2 McFarland standard, since with a higher density some isolates of *N. gonorrhoeae* produced stringy suspensions which made reading of reactions difficult. In such instances, retesting after the addition of sufficient TH to the boiled suspension to bring the density down to a no. 2 McFarland standard resulted in a satisfactory reaction. The speed and intensity of reactions of *N. gonorrhoeae* in TH-PMGOT were similar to those in GG, possibly because a heavier suspension could be used without the risk of false-positive results, but because of the colored antigen used in the test, the reactions in PMGOT were easier to read. According to Dillon et al. (4), the PMGOT kit is the least expensive of the commercially available serologic confirmation tests; therefore, TH-PMGOT offers a convenient and economical alternative procedure for the confirmation of *N. gonorrhoeae*.

Because an occasional isolate fails to react in any of these tests, laboratory technologists need to be highly suspicious when colonies with a morphology typical of *N. gonorrhoeae* fail to react in them and to test such isolates with an alternative system.

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