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Panel of Reference Strains for Evaluating Serologic Reagents Used To Identify Gonococci

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A panel of strains for evaluating *Neisseria gonorrhoeae* serologic reagents was developed. The strains selected for the panel were antigenically diverse and representative of strains isolated worldwide and had been isolated from a variety of anatomic sites. A few strains with characteristics that can cause problems in serologic tests were included. The panel of 52 gonococcal and 20 nongonococcal strains was used to evaluate two commercially produced kits with monoclonal antibody reagents, GonoGen and Phadebact, and one Phadebact kit with absorbed rabbit antiserum. The GonoGen reagent correctly identified all gonococcal strains and did not react with any of the nongonococcal strains. The Phadebact absorbed antiserum reagent correctly identified 47 of 48 gonococcal strains but reacted with 2 of the 20 nongonococcal strains. The Phadebact monoclonal antibody reagent correctly identified all of the gonococcal strains; however, it gave positive reactions with 8 and trace reactions with 4 of the 20 nongonococcal strains.

In evaluating diagnostic reagents, clinical studies serve an important function but have limited value in that they usually involve only one lot of the reagent and the strains tested are representative of those isolated in a particular geographic area at a particular time. Thus, in a clinical study, a reagent or kit could be inadequately tested for both homologous and heterologous reactions. For use in evaluating serologic reagents that identify *Neisseria gonorrhoeae*, we developed a panel of test strains selected from collections worldwide and with specific characteristics. We used these to determine the efficacy of three commercially produced kits for identifying gonococci.

MATERIALS AND METHODS

The strains selected for the test panel are listed in Tables 1, 2, and 3 and were obtained from the *Neisseria* Reference Laboratory (NRL), University of Washington, Seattle, unless indicated otherwise. References and information pertinent to the strains in Table 1 are presented elsewhere (4), and information relevant to the other strains is given in Tables 2 and 3.

In preparation for lyophilization, all cultures were grown for 16 to 18 h at 37°C in 5% CO_2 in a humidified incubator on GC agar base (BBL Microbiology Systems, Cockeysville, Md.) with the supplements described by Kellogg et al. (6) and checked for purity and growth. Growth for lyophilization was suspended in a mixture of 5% *myo*-inositol with newborn calf serum.

Samples of each lyophilized culture were rehydrated with 5 or 6 drops of Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) and cultured as described above. The identity of all the isolates was reconfirmed by personnel of two laboratories at the Centers for Disease Control. The following tests were performed: Gram stain, oxidase, Superoxol (Merck & Co., Inc., Rahway, N.J.; catalase test with 30% hydrogen peroxide), and rapid micro-carbohydrate utilization (16). In addition, gonococci were serogrouped by coagglutination with monoclonal reagents supplied by the NRL (7, 13). Lyophilized cultures were coded and held at 4°C. Before they were used to evaluate the commercially produced reagents, the cultures were rehydrated and grown as before, followed by subculture on modified Thayer-Martin (MTM) medium (8a). MTM medium was the preferred medium named in the package insert dated January 1984 that accompanied the Phadebact gonococcus test. In the GonoGen instructions (February 1984), no preferred medium was given. Subcultures on MTM medium were used to test two of the commercially produced reagents, except that growth from the supplemented GC agar was used for six cultures that did not grow on MTM medium (*Neisseria mucosa*, *N. flava*, *N. elongata* subsp. *glycolytica*, *N. gonorrhoeae*, and two strains of *N. subflava*). The Phadebact monoclonal antibody kit was tested with all cultures grown on GC agar, which was the medium suggested in the package insert dated May 1985. This kit was marketed shortly after we tested the other products.

We evaluated the Phadebact gonococcus test (lot no. 8854) and the Phadebact monoclonal GC OMNI test (lot no. 1582), both made by Pharmacia, Uppsala, Sweden, and the GonoGen test kit (lot nos. 122985 and 102786) made by New Horizons Diagnostics Corp., Columbia, Md. GonoGen (lot no. 122985) was used to test all strains except for five *N. gonorrhoeae* cultures that were tested with lot no. 102786. Phadebact kit lot no. 8854 used absorbed rabbit antiserum in a coagglutination test. The Phadebact monoclonal OMNI test and the GonoGen test each contained a reagent consisting of monoclonal antibodies to protein I of *N. gonorrhoeae* bound to nonviable staphylococci and a reagent consisting of normal rabbit antibody bound to staphylococci. In addition to these, the GonoGen kit had positive and negative control reagents. Glass slides were provided with the GonoGen kit, and disposable slides were provided with the Phadebact monoclonal antibody kit.

The manufacturers' instructions stated that only those organisms presumptively identified as *Neisseria* spp. (oxidase-positive, gram-negative diplococci) were suitable for testing. We used the "colony sample procedure" described in the package insert to test the Phadebact kit lot no. 8854. Approximately 10 to 15 colonies were taken from MTM medium and smeared onto a slide. A drop of gonococcal reagent was mixed with the colonies. Positive reactions were read as soon as they appeared; negative reactions were read

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TABLE 1. Serologic reference strains of *N. gonorrhoeae* that represent the antigenic diversity of gonococcal protein IA and IB molecules and were used to evaluate diagnostic reagents

NRL no. (synonyms)	MOMP[a] class	W serogroup[a]	POMP[b] serotype	MIF[c] type
32775[d] (7926, 8038, 5293)	A-1	II	8	B1
32776 (7876)	B-2	I		
32777 (7875)	C-3	II		
32778 (7877)	D-4	I		
32779 (7878)	E-5	I		
32780 (7879)	F-6	III		
32781 (7873)	G-7	I		
7870	H-8	II		
32784 (7872)	N-10	II		
32785 (7871)	R-11	I		
32786 (7874)	S-12	II		
32787 (7924)	T-13	II		
6611[e] (7928, 32788)	U-14	II	4	
32789 (7925)	V-15	I		
32790 (7929)	W-16	I	3	
32783 (7927)	X-9	I		
8658		I	2	
7122		I	1	
8658		I	2	
5767		II	5	
8035		II	6	
5766		II	7	
8660		III	9	
4286				A1
1859		I		A2
1567		I		A3
5288		II		B2
5001				B3
5016				C1
1955				C2

[a] The major outer membrane protein (MOMP) reference strains (NRL 7870 to 7879 and 7924 to 7929) were submitted to the NRL by K. H. Johnston (5). These strains were used as prototype strains for coagglutination W serogrouping by Eric Sandström (12) and were resubmitted to the NRL (NRL 32775 to 32790). In addition to strains listed in the left-hand column, strains 5293, 7929, and 8038 were also used to evaluate diagnostic tests.

[b] POMP, Principal outer membrane protein antigens (3).

[c] MIF, Microimmunofluorescence (14).

[d] Strain F62 was obtained by the NRL from four sources and designated NRL 5293, 7926, 8038, and 32775, respectively.

[e] Strain 33 was received by the NRL from three sources and designated NRL 6611, 7928, and 32788, respectively.

within 2 or 3 min. Specimens that gave noninterpretable results were retested by the "boiled colonies suspension" protocol using a heavy suspension of organisms in 0.2 ml of distilled water. In the Phadebact monoclonal antibody procedure, a light suspension of organisms in 0.5 ml of 0.9% saline was required. The term "light suspension" was not defined in the instructions for the product.

In the GonoGen procedure, we used a thick suspension of organisms (McFarland no. 3 or 10^8 organisms per ml) in 0.2 ml of distilled water as described in the package insert. Suspensions were covered and heated in a boiling-water bath for 5 min in the Phadebact tests and 10 min in the GonoGen test. The Phadebact monoclonal antibody test results were read within 1 min, and the GonoGen results were read within 2 min.

RESULTS

The Phadebact absorbed antiserum kit correctly identified 47 of the 48 *N. gonorrhoeae* strains tested. The kit failed to identify strain NRL 33520 (Table 2). Four strains, NRL 36022, 36023, 36024, and 32314, were received after the expiration date of this kit and were not tested. Eight of the *N. gonorrhoeae* strains, NRL 7871, 7924, 5766, 5001, and 37720 and CDC 85-022252, 85-020738, and 85-020737, gave noninterpretable reactions by the colony sample procedure and were identified by the boiled colonies suspension protocol. The Phadebact monoclonal OMNI reagent reacted correctly with each of the 52 *N. gonorrhoeae* strains.

The GonoGen kit correctly identified 52 of 52 *N. gonorrhoeae* strains. Strains NRL 32790 (Table 1), 37717, and 37718 (Table 2) gave weak-positive reactions with the GonoGen reagent.

Only the GonoGen kit reacted correctly with all of the 20 nongonococcal strains in Table 3. With these strains, the Phadebact absorbed antiserum kit gave two incorrect results and the Phadebact monoclonal antibody kit gave eight incorrect positive results plus trace reactions with four strains.

DISCUSSION

In evaluating three serologic tests for rapid laboratory diagnosis of *N. gonorrhoeae*, we designed a panel of reference gonococcal strains that represented the diverse protein I serogroups and serovars described previously (Table 1) (3, 5, 8, 12, 14). Most serovars shared common epitopes, so that one isolate could be used to represent a group of antigenically related serovars (8). The panel was supplemented (Table 2) with isolates that were well documented in the literature, that were representative of African and Asian strains, and that represented serovars that would not have reacted with a reagent composed of several monoclonal antibodies (10) and serovars that were not detected in a previous evaluation of GonoGen (9).

Strains representative of other *Neisseria* spp. and related bacteria were also included in this panel. The nongonococcal isolates resemble *N. gonorrhoeae* either in Gram stain, cell morphology, and oxidase reaction, may grow on selective medium for isolating *N. gonorrhoeae*, or may be isolated on nonselective medium from anatomic sites that would normally be sterile. One species, *Neisseria lactamica*, was reported to react with the Phadebact gonococcus test (11), and strains of *Neisseria cinerea* have been found to react with this reagent (Knapp; personal communication). Isolates of these and other nongonococcal species reacted with the Phadebact OMNI reagent.

Both the *N. elongata* subsp. *glycolytica* and the *N. lactamica* strains misidentified as *N. gonorrhoeae* with the Phadebact absorbed antiserum reagent were isolated from nongenital sources. According to Phadebact instructions accompanying this reagent, if a nongenital isolate is coagglutination positive an *o*-nitrophenyl-β-D-galactopyranoside test should be done. This would avoid the erroneous result on the *N. lactamica* strain but not the *N. elongata* strain. The package insert for the Phadebact OMNI test gave no instructions to do additional testing of nongenital isolates, and all positive strains were reported as *N. gonorrhoeae*.

Each product tested was accompanied by instructions describing the types of cultures for which the test was appropriate and conditions for correct test performance. A specific problem encountered with the Phadebact OMNI test was the instruction, without further elaboration, to use a "light suspension." The Phadebact OMNI test differed from the other gonococcal coagglutination tests, which required use of a "heavy suspension." The manufacturer of the

TABLE 2. Additional *N. gonorrhoeae* strains used to evaluate diagnostic reagents

NRL no.	Sender, strain designation[a]	Geographic source	Yr isolated	Pertinent information
37716	GE, GC 340/V1·RA, GC 340/V1	Georgia	1978	Distinct immunotype (15)
37717	GE, GC 1931V2·RA, GC 1931/V2	Georgia	1978	Distinct immunotype (15)
37720	GE, 2686·RA, 2686	Georgia	1972	Chimpanzee avirulent (2)
33520	PP, 82.057114	Ghana	1982	Cervical isolate, β-lactamase negative
33525	PP, 82.057126	Ghana	1982	Cervical isolate, β-lactamase positive
7786	PP, CDC206-2-5-R	Philippines	?	β-Lactamase positive
9718	PP, 77.021972	Philippines	1976	β-Lactamase positive
37718	GE, 78.071113·RA, GC 113	Alabama	1978	Detection of specific agglutination difficult due to extracellular DNA (1)
37719	GE, 78.072092·RA, GC 092	Pennsylvania	1978	See comment for NRL 37718
30010	Unknown	?	?	ATCC[b] 19424[T] (NCTC[c] 8375)
36022	JK·BM	Seattle	1983	COA[d] serovar IA-4 (9)
36023	JK·BM	Seattle	1983	COA serovar IA-4 (9)
36024	JK·BM	Seattle	1983	COA serovar IA-4 (9)
32314	JK	Seattle	1981	COA serovar IB-29; reacts only with monoclonal antibody reagent 2D6 (unpublished data)
	GE, 85.022252·JB, 85.022252	?	?	COA serovar IA-4
	GE, 85.020143·JB, 85.020143	New Mexico	1985	COA serovar IB-2, β-lactamase negative
	GE, 85.020738·JB, 85.020738	Georgia	1985	COA serovar IB-2, β-lactamase positive
	GE, 85.000945·JB, 85.000945	Georgia	1985	COA serovar IB-2, β-lactamase negative
	GE, 85.020737·JB, 85.020737	Georgia	1985	COA serovar IB-2, β-lactamase positive
	GE, 85.000818·JB, 85.000818	Georgia	1985	COA serovar IB-14, β-lactamase negative

[a] The strain pedigree is presented in the following manner: GE, GC340/V1•RA, GC340/V1 indicates that Robert Arko supplied strain GC340/V1 to Gracia Evins, who cataloged the strain under the same number, GC340/V1. When this strain was received in the NRL, it was cataloged as NRL 37716. Abbreviations: AR, Alice Reyn; GE, Gracia Evins; RA, Robert Arko; PP, Peter Perine; JK, Joan Knapp; BM, Barbara Minshew; JB, James Biddle.
[b] ATCC, American Type Culture Collection, Rockville, Md.
[c] NCTC, National Collection of Type Cultures, Colindale, London, England.
[d] COA, coagglutination.

TABLE 3. Reactions of three gonococcus test kits with nongonococcal strains of *Neisseria* spp. and strains of *Branhamella catarrhalis*, *Kingella denitrificans*, and *Moraxella osloensis*

Test reaction			Species	Strain[a]	Additional information and other strain designations[b]
Phadebact		GonoGen monoclonal antibody			
Absorbed antiserum	Monoclonal antibody				
−	+	−	*N. cinerea*	NRL 30003	Human nasopharynx; ATCC 14685[T], Berger 194
−[c]	+	−	*N. elongata*	NRL 30006	Human nasopharynx; ATCC 25295[T], Holten M2, NCTC 10660
+	Trace	−	*N. elongata* subsp. *glycolytica*	NRL 30007	Throat swab; ATCC 29315[T], Henriksen 6171/75, NCTC 11050
−[c]	+	−	*N. flava*	NRL 30008	Berger 10d
−	Trace	−	*N. flavescens*	NRL 30009	Spinal fluid; ATCC 13120[T], Branham N155
+	+	−	*N. lactamica*	NRL 30011	Nasopharynx; ATCC 23970[T], Hollis NCDC A7515, NCTC 10617
−	−	−	*N. lactamica*	RA, F2850	Pernasal isolate
−	+	−	*N. lactamica*	JB, D-1748	Genital isolate
−	Trace	−	*N. meningitidis*	NRL 30012	Spinal fluid; ATCC 13077[T], Branham M1027, NCTC 10025
−	+	−	*N. meningitidis*	RA, F2808	Maltose positive
−	+	−	*N. meningitidis*	RA, Mal[−]	Maltose negative
−	−	−	*N. mucosa*	NRL 30013	Sputum; ATCC 19696[T], Véron N16 (CIP 5951)
−	−	−	*N. sicca*	NRL 30016	Pharyngeal mucosa; ATCC 29256, Berger 6b
−	−	−	*N. subflava*	NRL 30015	ATCC 10555, Branham 7078 (*N. perflava*)
−	+	−	*N. subflava*	NRL 30017	Berger H231
−	−	−	*B. catarrhalis*	NRL 30018	ATCC 25238, Catlin Ne 11
−	Trace	−	*B. catarrhalis*	RA, 81.064126	Grows on MTM medium
−	−	−	*K. denitrificans*	RA, E1399	Grows on MTM medium; normal pharyngeal flora; occasionally isolated from rectal and genitourinary sites and blood
−	−	−	*K. denitrificans*	RA, E2281	See note for strain E1399
−	−	−	*M. osloensis*	DH, E398	May appear similar to *N. gonorrhoeae* in a Gram-stained smear

[a] Abbreviations: ATCC, American Type Culture Collection; NCTC, National Collection of Type Cultures, London; CIP, Collection of the Pasteur Institute, Paris; RA, Robert Arko; JB, James Biddle; DH, Danny Hollis.
[b] Information from the ATCC Catalog of Strains, 1980, 14th ed., or from the individuals who provided the strains.
[c] Reaction not interpretable when tested by the colony sample procedure and was negative by the boiled colonies suspension protocol.

Phadebact OMNI test now defines a light suspension as equivalent in density to a McFarland 0.5 standard.

By using as a test panel reference strains of the four serologic classification systems given in Table 1, we obtained much greater strain diversity than we may have encountered with clinical isolates from one geographic area. In addition, these strains were isolated from a variety of anatomic sites.

Strains chosen for the nongonococcal testing panel were those likely to cause problems in the clinical laboratory. Like *N. gonorrhoeae*, many of these strains will grow on MTM medium, are gram-negative diplococci or coccobacilli, and are oxidase positive. In addition, many of these organisms commonly inhabit the nasopharynx and may occasionally be isolated from the urogenital tract.

This panel of strains was devised to use in lot-to-lot testing of commercially produced *N. gonorrhoeae* serologic reagents. Since these products are used world wide, it was important to have as serologically diverse a set of strains as possible and yet also limit the number of strains. The prevalence of particular serovars varies geographically and temporally; a panel of reference strains should be independent of these changes. This panel of strains may require modification to reflect important changes in gonococcal strain populations. Strains included in the panel should represent clinically important isolates, including antimicrobial agent-resistant strains such as spectinomycin-resistant isolates.

It is important to remember that this panel of strains was selected on the basis of the diversity of epitopes on an outer membrane protein, protein I. This panel may not adequately represent gonococci with respect to other cell components including other proteins or lipopolysaccharide. It is also possible that, although this panel of strains may adequately represent *N. gonorrhoeae*, changes may be warranted with time to adequately represent newly emerging antibiotic-resistant strains and strains from patients with unusual syndromes.

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