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Comparison of GonoGen, GonoGen II, and MicroTrak Direct Fluorescent-Antibody Test with Carbohydrate Fermentation for Confirmation of Culture Isolates of *Neisseria gonorrhoeae*

J. A. KELLOGG* AND L. K. ORWIG

Clinical Microbiology Laboratory, York Hospital, York, Pennsylvania 17405

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When testing 248 clinical isolates of *Neisseria gonorrhoeae*, the sensitivity was 100% with GonoGen (Becton Dickinson Microbiology Systems), 99.6% (247 of 248) with GonoGen II (Becton Dickinson), 97.2% (241 of 248) with the MicroTrak direct fluorescent-antibody test (Syva), and 97.6% (242 of 248) with Rapid Fermentation Agar carbohydrates (Remel). Of 62 isolates of other *Neisseria* species, none was misidentified as *N. gonorrhoeae* by GonoGen, MicroTrak, or Rapid Fermentation Agar carbohydrates but 7 (31.8%) of 22 isolates of *N. meningitidis* gave strong, repeatedly false-positive results with GonoGen II. The sensitivity of all four assays was good to excellent, but all positive GonoGen II results should be confirmed with an independent assay, especially when isolates are recovered from sites where *N. meningitidis* is likely. Positive results from any of the assays should be routinely confirmed when dictated by specific clinical, legal, or microbiological circumstances.

For medical, social, and legal reasons, it is becoming increasingly necessary to confirm a clinical isolate identified as *Neisseria gonorrhoeae* by using a second test (3, 4, 13, 14, 23, 26). For decades, the standard method of identification of the pathogen has been acid production from cystine tryptic agar digest medium containing carbohydrates (2, 14). However, false-positive carbohydrate results have been reported due to contamination of the inoculum with other bacteria or of one carbohydrate with another fermentable carbohydrate (9, 14, 15, 25). False-positive results have also occurred because of incubation of the assay tubes in a CO_2 atmosphere (14). In addition, false-negative carbohydrate results have been reported due to slowly growing isolates, strain variation, the use of an insufficient or old inoculum; inadequate incubation conditions, including time (3 days or more may be required), humidity, or temperature; and a pH reversion in the cystine tryptic agar medium (3, 6, 7, 9, 13–16, 18, 27).

Monoclonal antibody tests are widely used to identify isolates of *N. gonorrhoeae* because of their speed and the relative accuracy of their results. However, false-negative results with direct fluorescent-antibody (DFA) (1, 8, 13, 22, 23) and coagglutination (3, 11, 12, 20) have been reported. Gonococci can rapidly produce changes in their outer membrane protein (the target for monoclonal antibody tests; 5, 10, 13, 14, 20, 25), resulting in false-negative DFA or coagglutination results (1, 13, 17). False-positive results have been reported less frequently with these reagents, although one false-positive result with GonoGen for an isolate of *N. meningitidis* has been reported (3). Some nonspecific Fc binding of monoclonal antibody reagents to staphylococcal cells may occur (26).

It has been suggested that identification of an isolate as *N. gonorrhoeae* by one method be confirmed with a test employing a different principle (biochemical, serological, RNA detection, etc; 14, 26). Because of ongoing concerns about the sensitivity and specificity of commonly used tests for identification of the pathogen, the performance of the GonoGen coagglutination and GonoGen II membrane immunoassay monoclonal antibody tests (Becton Dickinson Microbiology Systems, Cockeysville, Md.), the Microtrak monoclonal DFA test (Syva, Inc., Palo Alto, Calif.), and Rapid Fermentation Agar (RFA) tubes containing 2% carbohydrate (Remel, Lenexa, Kans.) were investigated with clinical isolates of *N. gonorrhoeae* and other *Neisseria* species. All three serological methods for identification of *N. gonorrhoeae* utilize antibodies prepared against purified outer membrane protein I of the species (3, 5, 10).

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Specimen collection and testing. Throat, genital, and rectal specimens for recovery of *N. gonorrhoeae* were collected with rayon-tipped Culturette swabs (Baxter Diagnostics Inc., Deerfield, Ill.). *Neisseria* species recovered on cultures from other body sites were also included in the study. Specimens for recovery of *N. gonorrhoeae* were immediately inoculated by the collector onto both Martin-Lewis and chocolate agar plates. The streaked cultures were incubated at 35°C in a 5 to 10% CO_2-enriched, humidified atmosphere for 3 days with daily inspection. All of the isolates used in the study were fresh clinical isolates of *Neisseria* species. Stock cultures were not tested because of a concern that they might give false-negative or delayed-positive test results which might not be expected from fresh clinical isolates.

When colonies suspected of being *N. gonorrhoeae* or another *Neisseria* species were observed, both a Gram stain and an oxidase tests were performed (14). Pure 18- to 24-h cultures of oxidase-positive, Gram-negative diplococci were then used to inoculate individual RFA tubes containing 2% glucose, maltose, sucrose, lactose, and fructose in accordance with the manufacturer's instructions. The RFA cultures were incubated aerobically for up to 24 h. A change of the phenol red pH indicator from red to yellow indicated utilization of a carbohydrate. Individual colonies were also tested with the GonoGen, GonoGen II, and MicroTrak DFA reagents as specified by the manufacturers. DFA slides were examined at a magnification of ×400 with an American Optical fluorescence microscope with a 100-W tungsten-halogen lamp and an excitor filter for fluorescein isothiocyanate. An isolate of oxidase-positive, Gram-negative diplococci was determined to be *N. gon-*

* Corresponding author. Mailing address: Clinical Microbiology Laboratory, York Hospital, 1001 S. George St., York, PA 17405. Phone: (717) 851-2393. Fax: (717) 851-2707.

TABLE 1. True- and false-positive results obtained with monoclonal antibody and carbohydrate fermentation tests for identification of *N. gonorrhoeae*

Species (no. of isolates)	No. (%) of isolates with positive reactions			
	GonoGen coagglutination	GonoGen II membrane immunoassay	MicroTrak DFA	RFA sugars
N. gonorrhoeae (248)	248 (100)	247 (99.6)	241 (97.2)	242 (97.6)[a]
N. meningitidis (22)	0	7 (31.8)	0	0
Other *Neisseria* spp. (40)	0	0	0	0[b]

[a] A true-positive result for identification of *N. gonorrhoeae* was acid production only in glucose.
[b] No nongonococcal isolate produced acid only in glucose.

TABLE 2. Frequency of need to repeat tests to obtain a true-positive or true-negative result for identification of *N. gonorrhoeae*

Result	No. (%) of isolates for which a test had to be repeated/total no. of isolates			
	GonoGen coagglutination	GonoGen II membrane immunoassay	MicroTrak DFA	RFA sugars
True positive	1/248 (0.4)	2/247 (0.8)[a]	3/241 (1.2)	27/242 (11.2)[b]
True negative	3/62 (4.8)	0/55 (0)	0/62 (0)	0/62 (0)

[a] Although only 2 of 247 GonoGen II true-positive results required test repetition before becoming positive, there were 7 repeatedly false-positive results with this method.
[b] Tests were repeated because of either false-negative glucose or false-positive RFA sugar results due to contamination.

orrhoeae if results of any three of the four (three serological and one biochemical) identifying tests so indicated and to be *N. meningitidis* if acid was produced only in glucose and maltose. All isolates of *N. meningitidis* grew on Martin-Lewis agar, and most reacted with serogroup-specific agglutinating antisera (Difco Laboratories, Detroit, Mich.). Discrepancies in the identification of isolates to the species level by any method were confirmed by repeating the appropriate tests. All of the identification systems used during the study were purchased by the York Hospital laboratory.

After 10 min of incubation of gonococcal isolates in GonoGen II solubilizing buffer-antibody reagent, 3 drops of this suspension were added to chocolate agar plates which were then streaked and incubated aerobically (with 5 to 10% CO_2) at 35°C for 2 days. This step was performed to determine if the buffer-antibody solution reliably killed the pathogen, because strips containing unused wells were stored for use later that day or on subsequent days. β-Lactamase activity was determined with a chromogenic cephalosporin assay (Cefinase; Becton-Dickinson) (1, 13, 14). A 95% confidence interval for each single-point proportion estimate was calculated as described by Wassertheil-Smoller (24).

Performance of tests for identification of *N. gonorrhoeae*. From 28 January 1993 until 30 June 1994, *N. gonorrhoeae* was recovered from 261 (5.8%) of 4,460 specimens. Of these isolates, 248 (95.0%) were tested with all four assays and were therefore entered into the study. The remaining 13 (5%) could not be completely tested, so their results were not included. Of the 248 isolates of *N. gonorrhoeae*, 2 (0.8%) were recovered from throat swabs, 13 (5.2%) were recovered from urine specimens, and 233 (94.0%) were found in cultures from genital specimens.

Of the four methods for identification of *N. gonorrhoeae*, only the GonoGen coagglutination test had neither false-positive nor false-negative results (Table 1). However, tests had to be repeated to obtain 0.4 to 1.2% of the true-positive results obtained with the serological reagents and 11.2% of the true-positive results found with the RFA sugars (Table 2). Repetition of the RFA tests was necessitated primarily by use of an inadequate inoculum (a large inoculum is required) and less frequently by a contaminated inoculum. With GonoGen, 3 (4.8%) of the true-negative results were obtained only after test repetition. The sensitivity of each assay, along with its specificity (with the exception of that of GonoGen II), ranged from good to excellent, with GonoGen performing the best (Table 3). Of seven false-negative results obtained with the MicroTrak DFA, 4 (57%) were associated with β-lactamase-positive isolates of *N. gonorrhoeae*. The one false-negative result found with GonoGen II came from a β-lactamase-negative isolate. Of 62 nongonococcal *Neisseria* species isolates tested, 7 (11.3%) gave repeatedly false-positive results with GonoGen II. All came from isolates of *N. meningitidis* (Table 1). False-positive GonoGen II results were encountered with four of the six lots of the assay that were tested during the study. In addition, GonoGen II solubilizing buffer and antibody did not always rapidly kill isolates of *N. gonorrhoeae*. Live *N. gonorrhoeae* was recovered on chocolate subculture plates from 16 (6.4%) of the 248 isolates after 10 min of incubation of the isolates in the reagent.

In the current study, the sensitivities and specificities of the GonoGen and MicroTrak DFA tests were similar to those found in previous studies. Very little has been reported for either the GonoGen II assay or RFA sugars. The reported sensitivity of GonoGen has ranged from 79 to 99.1% in early studies (11, 12, 20) and from 99.1 to 100% with improved reagents (3, 5). The reported sensitivities of GonoGen II, MicroTrak DFA, and CTA carbohydrates were 98.6% (8), 94 to 100% (1–3, 8, 10, 19, 25), and 60.9 to 99.1% (3, 15, 16, 18, 25, 27), respectively. Strains of *N. gonorrhoeae* that resulted in false-negative DFA results have often (1, 8, 22, 23), but not always (1, 13), been reported to be β-lactamase positive. The reported specificities of GonoGen, MicroTrak DFA, and CTA carbohydrates were 99.1 to 100% (3, 5, 11, 20), 100% (2, 3, 8, 10, 19, 25), and 80.8 to 100% (3, 15), respectively. Maltose-negative strains of *N. meningitidis* exist and therefore could be misidentified as *N. gonorrhoeae* if a carbohydrate utilization assay were used to identify the isolates (6, 7, 13, 15, 21, 27). None of the 22 isolates of *N. meningitidis* in the current study was maltose negative. The high specificity (100%) previously reported for GonoGen II (8), despite the fact that the product was tested with 74 isolates of *N. meningitidis*, was not observed in the current study. This discrepancy in GonoGen II specificity between the two studies may represent lot-to-lot variation, as was observed in the current study.

The results of the present study support the previous recommendations for confirmation of the identity of isolates of *N.*

TABLE 3. Overall performance of tests for identification of *N. gonorrhoeae*

Test	% Sensitivity	% Specificity
GonoGen	100	100
GonoGen II	99.6 (98.8–100)[a]	88.7 (80.9–96.5)
Microtrak DFA	97.2 (95.0–99.4)	100
RFA carbohydrates	97.6 (95.6–99.6)	100

[a] The values in parentheses are 95% confidence intervals.

gonorrhoeae in specific clinical or microbiological circumstances, including cases of suspected child abuse (3, 4, 13, 14, 23, 26), recovery of an isolate from an extragenital site or a low-risk patient (14), or a hard-to-interpret result (14). In addition, all positive GonoGen II results should be confirmed, especially when isolates are recovered from sites where *N. meningitidis* is likely. Since the GonoGen II reagents did not always rapidly kill isolates of the pathogen, caution should be practiced when performing tests in wells on strips of this assay which have been previously used.

Statistical analysis of the results was performed by Sally H. Cavanaugh, Research Department, York Hospital.

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