Evaluation of a Urease-Based Confirmatory Enzyme-Linked Immunosorbent Assay for Diagnosis of *Neisseria gonorrhoeae*

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A new urease-based enzyme-linked immunosorbent assay utilizing novel monoclonal antibodies was evaluated for the culture confirmation of *Neisseria gonorrhoeae*, with 270 isolates of *N. gonorrhoeae*, 56 isolates of diverse *Neisseria* spp., and 29 *Moraxella* isolates. The test was highly specific (100.00%) and sensitive (97.83%). No cross-reactions were observed with any of the *Neisseria* or *Moraxella* isolates tested. Fifty percent (3 of 6) of the false-negative results were obtained with isolates of serovar IA-4, a serovar rarely encountered in North America.

*Neisseria gonorrhoeae* is one of the leading causes of sexually transmitted diseases (10, 20, 21). The accurate and expedient diagnosis of gonorrhea requires laboratory tests that are highly sensitive and specific. The traditional method for identifying *Neisseria* species was a carbohydrate degradation test using cystine-tryptic digest agar base medium (CTA sugars) (23, 27); this test requires 24 to 48 h of incubation. Recently, alternative rapid methods, including substrate (carbohydrate and chromogenic) utilization tests, various immunological tests (1, 2, 5, 11, 12, 16, 17, 22, 25, 28), and DNA probe tests (14, 19, 20, 24, 26), have been evaluated.

Several immunological methods, such as coagglutination and fluorescent-antibody tests, have been used as confirmatory tests after primary culture (2, 11, 12, 17). Enzyme immunoassays, such as Gonozyme, directly detect gonococcal antigens from clinical specimens, eliminating the need for primary culture but compromising the sensitivity and specificity of the test (16, 25). In order to maximize automation without hindering the sensitivity and specificity of the test, a unique urease-based sandwich enzyme-linked immunosorbent assay (ELISA) system (the ELISA G.C. kit) using previously developed monoclonal antibodies was developed (ADI Diagnostics Inc., Rexdale, Ontario, Canada). We report on the evaluation of this system for its ability to confirm the presence of *N. gonorrhoeae*.

A total of 355 strains were evaluated with the ELISA G.C. kit. These included 270 *N. gonorrhoeae* isolates, consisting of nine auxotypes and 14 serovars and 56 *Neisseria* isolates (33 *N. meningitidis* isolates belonging to nine serogroups, 9 *N. subflava* isolates, 5 *N. cinerea* isolates, 4 *N. lactamica* isolates, 3 *N. sicca* isolates, 1 *N. maccosa* isolate, and 1 *N. flavescens* isolate), and 29 *Moraxella* isolates (26 *Branhamella catarrhalis* isolates and 3 *Moraxella phenylpyruvica* isolates). The isolates were selected from the culture collection of the National Laboratory for Sexually Transmitted Diseases, Laboratory Centre for Disease Control, Ottawa, Ontario, Canada. This collection included 88 isolates collected by the National Laboratory for Sexually Transmitted Diseases during a 1988 to 1989 National Surveillance Study (8) and 182 isolates previously selected for the evaluation of different diagnostic kits and serotyping methods for *N. gonorrhoeae* (5, 11). All of the isolates had been stored either frozen at −70°C in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) plus 15% glycerol or lyophilized in 2% skim milk (9). Before testing, isolates were subcultured on GC medium base (Difco) supplemented with 1% Kellogg defined supplement and incubated overnight in a humid atmosphere with 5% CO₂. Isolates were initially identified or reconfirmed by Gram stain, oxidase reaction, colony morphology, and sugar utilization tests. The identities of isolates giving anomalous results were reconfirmed by immunological tests, GonoGen, Syva MicroTrak, and a carbohydrate utilization test, Minitek.

The two monoclonal antibodies (MAbs), NG-38 and NG-78, used in this capture assay (22) were obtained following the immunization of mice with lithium acetate-extracted outer membrane preparations (3, 22). Both MAbs are immunoglobulin G2a; NG-38 and NG-78 recognized only protein IB (PIB) and protein IA (PIA) strains of *N. gonorrhoeae*, respectively, as ascertained by dot immunoassay (22). These MAbs did not react with other *Neisseria* spp. or more distantly related bacterial species.

The sandwich ELISA kit, prepared at ADI, included antibody-coated microwell strips (12 × 1 mm), extraction buffer, monoclonal antibody conjugate, substrate solution, and positive and negative control solutions. The 12-well strips were precoated with both purified rabbit antibodies against *N. gonorrhoeae* PIA and PIB strains. Bacterial suspensions were prepared in 0.1 M phosphate-buffered saline (PBS), and their optical densities were measured at 470 nm. The suspensions were diluted in extraction buffer to a final concentration of approximately 5 × 10⁷ cells per ml or within a 10-fold range of this value. Antibody-coated strips were inoculated with the extracted samples (final concentration, 5 × 10⁷ cells per well) followed by 50 μl of urease-conjugated monoclonal antibody solution (6, 15). The posi-

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tive and negative controls consisted of individual outer membrane extracts (5 μg/ml) of PIA strain NLI-S2, PIB strain NLI-S3 of N. gonorrhoeae, and strain 604A of N. meningitidis. After the extracted bacteria and MAb conjugates were incubated for 30 min at 37°C, the plate was washed six times with 0.85% saline, the urease substrate solution was added, and the plate was incubated for 30 min at room temperature. The A620 of the product of the reaction was read. A result was defined as positive when the absorbance of the sample minus the mean absorbance of two negative controls was greater than the control absorbance plus a factor of 0.05. All testing was performed in a single-blind study format.

Of 276 isolates identified as N. gonorrhoeae by biochemical and immunological identification tests, the ELISA identified 270 and failed to identify 6. Of the 85 nongonococcal isolates identified by biochemical identification tests, the ELISA identified as negative all 85. The ELISA was 100% specific, as no other species were misidentified as N. gonorrhoeae. However, even after retesting, the immunoassay failed to identify six isolates of N. gonorrhoeae, thus contributing to a sensitivity of 97.83%. The positive predictive value was 100.00%, while the negative predictive value was 93.40%. All of the false-negative isolates were prototrophic; two belonged to serovar IB-1, one belonged to serovar IB-2, and three belonged to serovar IA-4. Difficulties in detecting isolates belonging to serovar IA-4 have been previously reported when the coagglutination method was used and when an enzyme immunosassay developed for the identification and serotyping of N. gonorrhoeae isolates was used (5, 22). Isolates belonging to serovar IA-4 are rare in North America (8, 18). In a recent Canadian national survey (1988 to 1989), only 0.2% of isolates belonging to IA serovars were typed as IA-4 (8). Other groups have reported this serovar to be common in isolates from geographical areas such as Sweden, where 45% of the penicillinase-producing N. gonorrhoeae isolates were serotyped as IA-4, and Scotland, which has 6% of all the PIA isolates belonging to this serovar (4, 7). The detection of such isolates and those reported to be nontypeable (15) may require the addition of an extra or additional monoclonal antibody.

With the ELISA kit, no false positives were detected with any of the nongonococcal isolates tested. By contrast, polyclonal antibody-based ELISAs for the direct screening of endocervical and urethral specimens, such as GONOZYME (Abbott), have a specificity ranging from 87.2 to 96.8% (16, 25). Periodic upgrading of ELISA kits to account for the genetic variation of protein I will ensure their reliability as confirmatory tests. Additional evaluations will be required to demonstrate the utility of this ELISA kit for the diagnosis of N. gonorrhoeae directly from urogenital samples. In conclusion, the present ELISA satisfied the criteria one looks for in a diagnostic kit: it was highly sensitive (97.83%) and specific (100.00%), easy to run, and economical (less than $2 per sample), allowing for the testing of a large number of samples, compared with coagglutination and fluorescence tests.

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