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Evaluation of Gonochek-II as a Rapid Identification System for Pathogenic *Neisseria* Species

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The Gonochek-II test kit (E-Y Laboratories, San Mateo, Calif.) may be used to rapidly identify (within 30 min) *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Neisseria lactamica*, and *Branhamella catarrhalis*. The kit consists of oxidase swabs, chromogenic substrates, and EY-20 reagent (diazonium salt derivative). A beta-lactamase tube is also included. Fifty-two clinical isolates were tested from cervical (22 isolates), urethral (13), throat (11), rectal (5), and urine (1) sources. All strains were oxidase-positive, gram-negative diplococci isolated on Thayer-Mayer plates incubated at 35°C in 5% CO_2 for 18 to 24 h. Each strain was tested by Gonochek-II, RapID NH (Innovative Diagnostics, Decatur, Ga.), and conventional rapid carbohydrate utilization. Forty-four isolates of *N. gonorrhoeae* and six *N. meningitidis* were identified. Only two isolates tested (4%) were identified as species other than these two. Gonochek-II outperformed all other methods for identification of *N. gonorrhoeae* from cervical and urethral areas. *N. meningitidis* strains were correctly identified in all cases. Ease and rapidity of the procedure, coupled with a small inoculum requirement and reliable results, led us to favor Gonochek-II for routine identification of pathogenic *Neisseria* species.

Traditional tests for the laboratory identification of pathogenic *Neisseria* species have been based on carbohydrate degradation reactions (1). A need for more rapid and reliable tests has led to the development of test kits which utilize enzymatic assays to differentiate these bacteria. In most of these systems, organism identification is achieved after several hours of incubation, followed by scoring of positive and negative reactions to give a profile specific for a particular species.

Recently, a test kit that uses mixed chromogenic substrates in a single test tube for the rapid (within 30 min) identification of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Neisseria lactamica*, and *Branhamella catarrhalis* has been introduced. E-Y Laboratories, San Mateo, Calif., has developed the Gonochek-II system to rapidly identify pathogenic *Neisseria* species. A glycosidase (beta-galactosidase) reaction and two aminopeptidase (gamma-glutamyl and proline aminopeptidase) reactions, which are mutually exclusive in the organisms to be tested and result in different colored end products, allow testing for several reactions in one tube. This significantly decreases the size of the inoculum required for organism identification. The inclusion of a rapid test for the presence of beta-lactamase permits the detection of penicillinase-producing strains of *N. gonorrhoeae*. Our purpose is to present the results of a comparison of the Gonochek-II test kit with the RapID NH System (2) and conventional rapid carbohydrate utilization tests (3).

MATERIALS AND METHODS

Organisms. Fifty-two clinical isolates from urethral, cervical, rectal, pharyngeal, and urine sources were obtained from cultures submitted to MicroDiagnostics and the Santa Clara Valley Public Health Laboratory. Suspicious colonies growing on modified Thayer-Martin plates (after incubation at 35°C in 5% CO_2) were tested for oxidase activity. Oxidase-positive organisms were Gram stained to confirm the presence of gram-negative diplococci. Each isolate was identified by Gonochek-II, RapID NH, and the conventional rapid carbohydrate utilization test (RCUT). The RCUT was considered the standard method. Subculture to one or two additional Thayer-Martin plates was usually required to ensure sufficient inoculum material for the three comparisons. Isolates were tested after approximately 18 to 24 h of incubation.

Gonochek-II procedure. Each test kit consists of an oxidase swab, a Gonochek-II tube (for species differentiation), a beta-lactamase tube (not used routinely in this laboratory), and a tube of EY-20 (diazonium salt derivative). Kits were removed from refrigeration and allowed to come to room temperature before use. Three drops of distilled water were dispensed into the Gonochek-II tube. Approximately 10 colonies were picked from the Modified Thayer-Martin plate with the wooden applicator stick provided and were emulsified into the Gonochek-II tube. The cap was replaced, and the tube was incubated for 30 min in ambient air at 35°C. EY-20 reagent was reconstituted with 1 ml of distilled water. After incubation, the color of the Gonochek-II tube was read. Table 1 shows the expected reactions and their interpretation. If the Gonochek-II tube was blue or yellow, the procedure was complete, and identification of *N. lactamica* or *N. meningitidis* could be made (Table 1). If there was no color change, one or two drops of reconstituted EY-20 reagent were added. A color change to pink-red resulted in an identification of *N. gonorrhoeae* (Table 1). No color change suggested the presence of *B. catarrhalis*.

RapID NH procedure. Conventional and single-substrate chromogenic tests in the RapID NH panel allow testing for phosphatase activity, nitrate reduction, *o*-nitrophenyl-β-galactopyranoside (ONPG) hydrolysis, proline aminopeptidase activity, 5-γ-glutamylaminopeptidase activity, resazurin reduction, glucose and sucrose utilization, indole production, ornithine decarboxylation, and urea hydrolysis. The inoculum for the RapID NH test kit consisted of a heavy suspension of the organism in the inoculum fluid, corresponding to a McFarland no. 3 nephelometry standard. The isolate was transferred to the fluid with a sterile cotton applicator, and the suspension was mixed well. The entire inoculum (1 ml)

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TABLE 1. Summary of biochemical reactions and results

Organism	Oxidase	Catalase	Gram stain	Gonochek-II reaction	Chemical principles
N. lactamica	+	+	GNDC[a]	Blue (after incubation)	Hydrolysis of β-D-galactoside bond by beta-galactosidase yields a blue color from the colorless substrate
N. meningitidis	+	+	GNDC	Yellow (after incubation)	Hydrolysis by gamma-glutamylaminopeptidase releases yellow *p*-nitroaniline from the colorless substrate
N. gonorrhoeae	+	+	GNDC	Red-pink (after incubation and addition of EY-20 reagent)	Hydrolysis of the beta-naphthyl amino acid derivative by proline aminopeptidase releases free beta-naphthyl amine derivative which complexes with EY-20 to produce a red-pink color
B. catarrhalis	+	+	GNDC	No color change (after incubation and addition of EY-20 reagent)	None of the above enzymes are present

[a] GNDC, Gram-negative diplococci.

was then added to the appropriate area of the panel. The individual microcupules were filled by tipping the panel to fill each cavity uniformly. After the panels were resealed, they were incubated in ambient air at 35°C for 4 h. After incubation and addition of reagents, each microcupule was examined for the presence or absence of a specific color change. After the test reactions were read and scored, the resulting codes were compared with the system data base in the code compendium to identify the isolate.

RCUT. The RCUT for differentiation of *Neisseria* species is based on acid production from carbohydrate degradation. The glucose, lactose, maltose and sucrose (Sigma Chemical Co., St. Louis, Mo.) solutions were prepared by E-Y Laboratories by the method of Young et al. (3). The phenol red indicator was included in the solutions, which were buffered and ready for use when purchased. Three drops of each sugar solution were dispensed into the respective test tubes and brought to incubation temperature (35°C) before inoculating them with organisms. A new applicator stick was used to inoculate each tube, and care was taken to break up any clumps of bacteria. A first reading was made after 30 min of incubation at 35°C. Positive reactions were indicated by a color change from red to yellow (or occasionally to yellow-orange). Normally, an identification was possible after 30 to 60 min, but a final reading could be made at 4 h if necessary.

RESULTS

Table 2 shows the sources of the 52 isolates, the oxidase and Gram stain results, and the identity of each isolate as determined by each technique. Forty-four isolates of *N. gonorrhoeae* and six of *N. meningitidis* were identified. The remaining two isolates were identified as *Neisseria subflava* and *N. lactamica*.

Of the *N. gonorrhoeae* isolates, four from cervical sources were nonreactive in the RCUT (Table 2). One cervical *N. gonorrhoeae* isolate was identified as *Kingella denitrificans* by triplicate RapID NH tests. Two *N. gonorrhoeae* isolates, one urethral and one rectal, were identified as *K. denitrificans* and *Kingella kingae*, respectively, by RapID NH. These same isolates were initially nonreactive in the RCUT. Repeat testing of both isolates with RapID NH and the RCUT resulted in an identification of *N. gonorrhoeae*, in agreement with the initial Gonochek-II results. One rectal and one throat *N. gonorrhoeae* isolate were initially missed by Gonochek-II, but correct identification was obtained on repeat testing.

Four *M. meningitidis* isolates from throats were identified as *Kingella kingae* by RapID NH. Another throat isolate was first identified as *N. gonorrhoeae* by RapID NH and RCUT. Repeat testing by all three methods confirmed the initial identification of *N. meningitidis* by Gonochek-II. The sixth *N. meningitidis* isolate was identified as *N. subflava* by RapID NH.

The *N. lactamica* isolate was initially misidentified by both the RCUT and Gonochek-II. Repeat testing by both methods resulted in correct identification.

The *N. subflava* isolate could not be specifically identified by Gonochek-II, although it was possible to rule out an identification of *N. gonorrhoeae* or *N. meningitidis* for this organism.

DISCUSSION

Our results indicate that the Gonochek-II system is a rapid and reliable method for confirmation of *N. gonorrhoeae* isolates. By using the RCUT as the standard method for identification of *N. gonorrhoeae*, the sensitivity and specificity of the Gonochek-II and RapID NH tests were compared. (The four specimens that were nonreactive on RCUT were not included in the statistical analysis.) On first-run results, the Gonochek-II system showed 95% sensitivity and 100% specificity (n = 48), whereas the RapID NH system was 92.5% sensitive and 87.5% specific (n = 48). On repeat testing, the Gonochek-II showed 100% sensitivity and 100% specificity (n = 48), whereas the RapID NH was 97.5% sensitive and 100% specific (n = 48).

The Gonochek-II system also outperformed the RapID NH system in confirmation of the six *N. meningitidis*

TABLE 2. Comparison of test results

Source (no. of isolates tested)[a]	Rapid sugars				Identification	
	Glucose	Maltose	Sucrose	Lactose	Gonochek-II	RapID NH
Cervix (17)	+	−	−	−	*N. gonorrhoeae*	*N. gonorrhoeae*
Urethra (12)	+	−	−	−	*N. gonorrhoeae*	*N. gonorrhoeae*
Urine (1)	+	−	−	−	*N. gonorrhoeae*	*N. gonorrhoeae*
Throat (2)	+	−	−	−	*N. gonorrhoeae*	*N. gonorrhoeae*
Rectum (3)	+	−	−	−	*N. gonorrhoeae*	*N. gonorrhoeae*
Cervix (4)[b]	−	−	−	−	*N. gonorrhoeae*	*N. gonorrhoeae*
Cervix (1)[c]	+	−	−	−	*N. gonorrhoeae*	*K. dentrificans* (nitrate interpretation)
Urethra (1)	+	−	−	−	*N. gonorrhoeae*	*K. denitrificans*,[d] *N. gonorrhoeae*[e]
Rectum (1)	+	−	−	−	*N. gonorrhoeae*	*K. kingae*,[d] *N. gonorrhoeae*[e]
Rectum (1)	+	−	−	−	*N. meningitidis*,[d] *N. gonorrhoeae*[e]	*N. gonorrhoeae*
Throat (1)	+	−	−	−	Non-*N. gonorrhoeae*,[d] *N. gonorrhoeae*[e]	*N. gonorrhoeae*
Throat (1)	+	+	−	−	*N. meningitidis*	*K. kingae*,[d] no identification[e]
Throat (1)	+	+	−	−	*N. meningitidis*	*K. kingae*
Throat (1)	+	+	−	−	*N. meningitidis*	*K. kingae*,[d] *N. meningitidis*[e]
Throat (1)	+	+	−	−	*N. meningitidis*	*K. kingae*
Throat (1)	+	+	−	−	*N. meningitidis*[d,e]	*N. gonorrhoeae*,[d] *N. meningitidis*[e]
Throat (1)	+	+	−	−	*N. meningitidis*	*N. subflava*
Throat (1)[f]	+	+	−	+	*N. gonorrhoeae*,[d] *N. lactamica*[e]	*N. lactamica*
Throat (1)[g]	+	+	+	−	Non-*N. gonorrhoeae*	*N. subflava*

[a] All isolates were oxidase-positive, gram-negative diplococci.
[b] Nonreactive in RCUT.
[c] RapID NH test run in triplicate producing identification of *K. denitrificans*; identified as *N. gonorrhoeae* by BACTEC; catalase positive.
[d] Identification on first run of test.
[e] Identification on repeat test.
[f] Lactose negative on initial RCUT; misread on initial Gonchek-II.
[g] Strongly catalase positive.

isolates tested. The *N. meningitidis* identification failures with the RapID NH system seemed to be related to difficulties with the detection of proline aminopeptidase or 5-γ-glutamylaminopeptidase activity, or both. The Gonochek-II system depends only on hydrolysis by gamma-glutamylaminopeptidase for the identification of *N. meningitidis*. Although the total number of *N. meningitidis* strains available for testing was small, we felt more comfortable with the confirmation of *N. meningitidis* strains by the Gonochek-II method.

We hesitate to comment in detail on the performance of Gonochek-II and RapID NH with strains other than *N. gonorrhoeae* and *N. meningitidis* owing to the limited number of such organisms identified in this study. Several strains from our stock collection were run on both Gonochek-II and RapID NH, including *B. catarrhalis* 155, *N. lactamica* ATCC 0032, *K. denitrificans* 153, and *Moraxella* sp. 154. Since none of these organisms can be differentiated from *N. gonorrhoeae* or *N. meningitidis* by the oxidase test alone, colonial and Gram-stain morphology, catalase test results, and growth patterns on Thayer-Martin media should be determined before biochemical confirmation of the isolate.

Identification of the *B. catarrhalis* strain was difficult with both RapID NH and Gonochek-II. The Gonochek-II reaction for *B. catarrhalis* is the same as that for an uninoculated tube. Inclusion of a nitrate test in the RapID NH panel should allow a more accurate identification of this organism, but we found the reaction difficult to interpret and were concerned about the possibility of false-negative interpretations.

The *N. lactamica* strain was identified correctly by both systems. A careful inspection of the Gonochek-II tube may be required to detect the blue color since addition of EY-20 reagent at this point gives erroneous identification.

To avoid misidentifications, catalase reaction, Gram-stain morphology, and growth characteristics on modified Thayer-Martin media should be carefully evaluated as indicated in the Gonochek-II package insert. The Gonochek-II system is not intended for identification of *Moraxella* or *Kingella* species. These organisms must be confirmed by another method since testing with Gonochek-II would give misleading results. The *Moraxella* strain appeared to be *N. meningitidis* by Gonochek-II since the tube was yellow after the incubation period. The *K. denitrificans* strain showed no color change in the Gonochek-II tube after incubation or addition of EY-20 reagent, indicating an identification of *B. catarrhalis*. The *Moraxella* strain could be differentiated from *Neisseria* species by the Gram-stain reaction (tiny gram-negative coccobacilli) and the inability to grow on modified Thayer-Martin agar. The *Kingella denitrificans* was a catalase-negative, gram-negative rod. (*Neisseria* species are catalase positive). When these strains were tested by RapID NH, the correct identifications of *K. denitrificans* and *Moraxella* sp. were obtained.

In conclusion, our results indicate that the Gonochek-II is a rapid, reliable method for confirming the identification of pathogenic *Neisseria* species. When many cultures are being screened exclusively for the presence of *N. gonorrhoeae*, as in our situation, the ability to obtain a rapid confirmation from the primary plate by a low-cost method such as

Gonochek-II provides savings of both time and money. We would reserve use of the more expensive and time-consuming RapID NH system for the identification of isolates other than pathogenic *Neisseria* species.

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