

Rapid Enzyme System for the Identification of Pathogenic *Neisseria* spp.

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Gonocheck II is a combination of three enzyme substrates in one tube which will give a rapid identification (30 min) of those pathogenic *Neisseria* spp. which can be isolated on Thayer Martin or similar selective media. Eighty isolates were tested by Gonocheck II and a carbohydrate utilization method; total agreement was achieved between the two methods.

A new method for the identification of pathogenic *Neisseria* spp. by using enzyme tests has recently become available (A. E. Chu, P. K. Chun, and D. M. Yajko, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C257, p. 279) (2). Gonocheck II is produced by E-Y Laboratories of San Mateo, Calif., and marketed in the United States by DuPont Co., Wilmington, Del. In the United Kingdom it is available from Tissue Culture Services, Slough, Buckinghamshire, England.

The test consists of a tube containing three substrates. (i) Beta-D-galactoside bond is hydrolyzed by beta-galactosidase to yield a blue color from the colorless substrate. Beta-galactosidase is produced by *N. lactamica*. (ii) Gamma-glutamyl-*p*-nitroanilide is hydrolyzed by gamma-glutamyl aminopeptidase to release yellow *p*-nitroaniline from the colorless substrate. Gamma-glutamyl aminopeptidase is produced by *N. meningitidis*. (iii) Beta-naphthyl amino acid derivative is hydrolyzed by prolyl aminopeptidase to release a free beta-naphthylamine derivative. This complexes with a diazonium salt called E-Y 20 reagent to produce a pink color. Prolyl aminopeptidase is produced by *N. gonorrhoeae*. *Branhamella catarrhalis* does not produce any of these enzymes.

We tested 80 strains of *Neisseria* isolated from the urethra, cervix, rectum, or throat of patients attending the Sexually Transmitted Diseases Clinic at The General Hospital in Birmingham. The isolation medium used was Columbia agar (Oxoid Ltd., Basingstoke, England), with 5% lysed horse blood (Tissue Culture Services) containing vancomycin (3.0 U/ml), colistin (7.5 µg/ml), and trimethoprim (3.0 µg/ml). A Gram stain was carried out on oxidase-positive colonies. Gram-negative diplococci were subcultured to obtain pure cultures for carbohydrate utilization tests. The Gonocheck II test was performed in most cases directly from the isolation plate.

The method used for carbohydrate utilization tests was a disk plate method (1). The medium was G.C. agar base with dried hemoglobin (Oxoid) and added IsoVitaleX (Becton Dickinson Ltd., Oxford, England). Four disks were used, containing maltose, lactose, glucose, and sucrose, respectively. The organism was spread evenly over the plate surface and incubated at 37°C for 4 h in a CO₂-enriched atmosphere produced by means of a candle jar. The disks were added, and incubation was continued for a further 20 h. The results were read by adding a drop of phenol red indicator to each disk, a positive reaction being indicated by

a definite yellow color change on the disk. Negative reactions produced a red disk.

Gonocheck II was used as recommended by the manufacturer. Four drops of distilled water were added to each Gonocheck II tube. Approximately 10 colonies were emulsified with a wooden applicator stick. The tube was incubated at 37°C for 30 min. A blue reaction in the Gonocheck II tube at the end of the incubation time indicated *N. lactamica*. A yellow reaction indicated *N. meningitidis*. If the tube was colorless, one drop of E-Y 20 reagent was added. A pink color indicated *N. gonorrhoeae*. If the tube remained colorless, it indicated *B. catarrhalis*.

The optimum pH for the gamma-glutamyl aminopeptidase reaction to produce a yellow color is 8.3. Our distilled water had a pH value of 6.8, and some yellow reactions were rather poor. In such cases, this could be improved by adding 2 drops of Tris buffer (pH 8.3) at the end of incubation. It was not recommended that this buffer be used as the test diluent. The literature from the supplier now contains the recommendation to use phosphate-buffered saline at pH 7.4 as the diluent.

Occasionally *Kingella* species may be encountered on selective media; thus, it is recommended that a catalase test be performed. *Kingella* species are catalase negative, whereas the *Neisseria* spp. and *Branhamella* spp. are catalase positive.

The following organisms were used to quality control the media and disks when the carbohydrate utilization tests were carried out and to quality control the batch of Gonocheck II used: *N. gonorrhoeae* NCTC 8375, *N. meningitidis* NCTC 10025, *N. lactamica* NCTC 10617, and *B. catarrhalis* NCTC 3622. At all times, these organisms gave the expected results with both the carbohydrate utilization tests and Gonocheck II. The batch of Gonocheck II which we used had a remaining shelf life of 6 months. Tests carried out on the expiration date produced correct results with the control organisms. Subsequent batches of Gonocheck II received have a shelf life of 10 months.

Of the 80 strains of *Neisseria* tested, 51 were identified as *N. gonorrhoeae*, 1 was identified as *N. lactamica*, and 28 were identified as *N. meningitidis*. Table 1 shows the identity and source of the isolates. There was total agreement between the Gonocheck II results and carbohydrate utilization tests. No strain of *B. catarrhalis* was isolated. We found the test easy to perform, and a rapid, accurate identification was achieved in all cases. Occasionally, there was insufficient growth on the isolation plate, and subcultures were made before testing. In most cases, identification was made

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TABLE 1. The identification of 80 strains of *Neisseria* species and their isolation sites

Isolation site	No. of strains identified by Gonochek II and carbohydrate utilization tests		
	<i>N. gonorrhoea</i>	<i>N. meningitidis</i>	<i>N. lactamica</i>
Urethra or cervix	33	1	0
Rectum	6	1	0
Throat	12	26	1

directly from the isolation plate, the result being available within 45 minutes of examining the plate.

Gonochek II should be used only on isolates from selective media. *B. catarrhalis* does not grow on our medium. However, if rare cases of this organism being isolated on selective media are reported, the test will identify these isolates. Nonselective chocolate agar should not be used for primary isolation, as other *Neisseria* species such as *N.*

sicca and *N. mucosa* may be isolated, and these will give misleading results.

We would recommend Gonochek II as a rapid, reliable, and easily performed method for the identification of pathogenic *Neisseria* spp. It would be of equal value to the laboratory only rarely isolating *Neisseria* spp., in which a reliable method with a long shelf life would be ideal, and to the laboratory serving a busy sexually transmitted diseases clinic, in which many such identification tests are performed each day.

LITERATURE CITED

1. Stacey, P., and G. Warner. 1973. Identification of *Neisseria gonorrhoeae* and *Neisseria meningitidis* by a carbohydrate disk technique. *J. Clin. Pathol.* **26**:303.
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