

## Correspondence

### Performance of tests for identification of *Neisseria gonorrhoeae*

Sir,

The identification of *Neisseria gonorrhoeae* by sensitive, precise, and rapid technique is important for the correct diagnosis and appropriate treatment of patients with gonorrhoea. For decades, the standard method of identification of the pathogen has been acid production from cystine tryptic agar (CTA) medium containing carbohydrates<sup>1</sup>. For a more rapid identification of this pathogen a variety of immunological and biochemical commercial kits have now become available. An ideal test should have high sensitivity and specificity, should be easier to perform and cheap. An attempt was made in this study to identify such a test by comparing four available tests on 71 *N. gonorrhoeae* isolates.

The three most widely used immunological kits are the MicroTrak *N. gonorrhoeae* culture confirmation test (an immunofluorescence test; Trinity Biotech PIC, Ireland), the Phadebact Monoclonal GC test (a coagglutination test; MKL Diagnostics AB, Kung Hans Vag, Sollentuna, Sweden) and GonoGen II (a membrane immunoassay; New Horizons Diagnostics, USA), all of which employ monoclonal antibodies (mAbs) developed against specific epitopes on the two types of the major outer membrane protein, protein I A and B (PIA and PIB)<sup>2</sup>. Interpretation of the MicroTrak test is less subjective but requires a fluorescence light microscope whereas Phadebact monoclonal test is easier to perform and can be used by most small laboratories<sup>2,3</sup>. GonoGen II is a colorimetric test and does not require heat treatment step of the coagglutination test. This test eliminates the reading of agglutination; instead, the mixture of antigen-antibody complex is passed through a filtration unit. The appearance of a red dot on the filter indicates a positive reaction<sup>2</sup>. Though the specificity of these kits for detection of *N. gonorrhoeae* is high, both false-positive (cross-reactions with other *Neisseria*

species such as *N. meningitidis*, *N. lactamica*, *N. cinerea* and *Kingella denitrificans*) and false-negative results have also been reported<sup>4</sup>.

The biochemical kits widely used are the *Neisseria* Preformed Enzyme Test (PET), Gonochek II, RapID NH, Neisstrip, API-NH and Rapid carbohydrate utilization test (RCUT)<sup>3-5</sup>. These tests are based on the presence of preformed chromogenic enzyme in the culture, and thus require a heavy inoculum of the organism grown on selective medium to permit rapid speciation of isolates. The enzymes that are detected by these systems include beta-galactosidase, gamma-glutamylaminopeptidase and prolyl-hydroxyprolyl aminopeptidase<sup>2</sup>. The *Neisseria* PET and Gonochek II are both single-use tubes containing chromogenic substrates that detect the presence of three preformed enzymes, each of which is indicative of a pathogenic *Neisseria* species, namely *N. lactamica*, *N. meningitidis* and *N. gonorrhoeae*. In Gonochek II, after incubation a red colour change (hydrolysis of L-proline 4-methoxynaphthylamide) is observed<sup>2,3</sup>. The API-NH and RapID NH kits employ a battery of tests, combining carbohydrate utilization and preformed enzymes. These two tests detect the change in prolyl iminopeptidase (PIP) enzyme activity due to the mutation in the *pip* gene. Not all isolates of *N. gonorrhoeae* possess PIP activity<sup>6,7</sup>. In RCUT, preformed enzyme is measured by adding a suspension of the overnight growth of the suspect organism to a buffered (non-nutrient) solution containing the sugar to be tested and a pH indicator. A yellow colour change indicated a positive reaction and orange red colour as negative reaction<sup>8</sup>.

As taxonomic differences between members of the *Neisseria* genus remain small, the identification of this pathogen can be problematic. Of the several commercial kits used worldwide for identification of

*N. gonorrhoeae*, only four commercial kits, RCUT, API-NH, Gonocheck II and Phadebact GC system were available in India. The purpose of this study was to compare the performance of these four commercial biochemical methods to identify pathogenic *N. gonorrhoeae* and to assess the cost effectiveness and utility in the clinical laboratory.

A total of 71 *Neisseria* isolates were included in the study carried out at the National AIDS Research Institute (NARI), Pune, India, during January 2007 to June 2009. Of these, 64 isolates of *N. gonorrhoeae* were obtained from patients attending STD clinics of NARI, Pune (n=16), Safdarjang hospital, New Delhi (n=35) and 13 collected under STI-Operations Research project conducted at NARI (Mumbai-6, Hyderabad-6 and Nagpur-1). Patients attending STD clinics with complaints of urethral/cervical discharge were included in the study. Seven non gonococcal *Neisseria* strains, *N. sicca* (n=2), *N. cinerea* (n=2) and *N. lactamica* (n=3) recovered from clinical specimens from patients attending NARI clinics were also included.

All the strains were tested by API-NH (Bio Merieux SA, France), Gonocheck II (E-Y Laboratories, CA, USA), Phadebact monoclonal GC OMNI test (Boule Diagnostics AB, Sweden) according to manufacturers' instructions, and by RCUT<sup>8</sup>. *N. gonorrhoeae* ATCC 49226 ( $\beta$ -lactamase negative) and WHO strains A, G H, E and O (received under WHO Gonococcal Antimicrobial Surveillance Programme from WHO GASP South East Asia Region Reference Laboratory, New Delhi) ( $\beta$ -lactamase positive) were used as quality control in each test run. The cost per test for each identification method was calculated considering the cost of media, reagents, ancillary supplies and time required to perform identification by each test method.

All isolates of *N. gonorrhoeae* were correctly identified with RCUT system and Phadebact

monoclonal GC OMNI system (Table). Gonocheck II test correctly identified in 68 (95.3%) of *N. gonorrhoeae* isolates. The low sensitivity of this test has also been reported earlier. API-NH system correctly identified 51 (79.6%) of *N. gonorrhoeae* isolates. Thirteen (20.3%) *N. gonorrhoeae* isolates did not show any reaction for PIP enzyme in the API-NH system, which could be done to the lack of enzyme in these isolates. Specificity of these tests was evaluated based on their ability to give negative results on the seven non gonococcal strains. RCUT system correctly identified *N. cinerea*, *N. sicca* strains and one of the three *N. lactamica* strains as non gonococcal strains. Similar findings were observed by Young and Moyes<sup>9</sup>. API-NH system correctly identified both *N. cinerea* strains, one of the two *N. sicca* strains and one of the three *N. lactamica* strains as non gonococcal strains. Gonocheck II test correctly identified one of the three *N. lactamica* strains as non gonococcal. Other studies have also reported similar findings in identifying *N. gonorrhoeae*, *N. lactamica* and *N. sicca* strains<sup>5,10</sup>. The Phadebact monoclonal GC OMNI system correctly identified both *N. cinerea* strains, two of the three *N. lactamica* strains and one of the two *N. sicca* strains. The sensitivity and specificity of Phadebact monoclonal GC OMNI system in our study was similar to that reported earlier<sup>5</sup>. Thus, RUCT appeared to be the most specific (6/7) followed by Phadebact OMNI system (5/7), and API-NH (4/7). Gonocheck II test showed lowest specificity by identifying six of the 7 non gonococcal strains as *N. gonorrhoeae*. RCUT was found to be the cheapest among all tests (Table).

The Phadebact monoclonal GC OMNI system was rapid, simple to perform, accurate and provided results in 2-3 min. However, it showed moderate specificity and was more expensive than other methods. The colours generated in the Gonocheck II system incubated at 30°C for 30 min were distinct, easy to read, stable

**Table.** Comparison of four test systems for the identification of *N. gonorrhoeae* isolates

Tests	Sensitivity* (%)	Specificity**	Approximate cost per identification (in ₹ )
API-NH	79.6	Low	400
Gonocheck II	95.3	Moderate	420
RCUT	100	High	100
Phadebact GC OMNI	100	Moderate	450

\*Sensitivity was estimated based on identification of 64 *N. gonorrhoeae* isolates

\*\*Specificity was graded qualitatively based on the ability of the test to identify seven non gonococcal strains as non gonococcal

and easy to handle in the clinical laboratory. However, it had low sensitivity (95.3%) in identification of *N. gonorrhoeae*. Additionally, it appeared to have low specificity since six of the seven non gonococcal strains were identified as gonococcal strains. API-NH system showed low sensitivity and specificity in identification of *N. gonorrhoeae*. This may be due to the lack of enzyme PIP in these isolates which have also been reported in other studies<sup>4,7</sup>. We found that the RCUT gave reliable results within 4 h, and identified 100 per cent of *N. gonorrhoeae* isolates as reported earlier by Yong and Moyes<sup>9</sup>. Additionally, it appeared to be highly specific since only one of seven non gonococcal strains was wrongly identified as gonococcal strain.

Considering the performance, rapidity and cost, RCUT appeared to be the best among the four methods studied for confirmatory identification of *N. gonorrhoeae*. RCUT can be used routinely as it is cheaper and easily available but the reagents used for RCUT should be evaluated with the known control strain before being used routinely.

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