Identification of *Neisseria gonorrhoeae* using the Neisstrip rapid enzyme detection test

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Abstract

A rapid enzyme activity strip test (Neisstrip, Lab M Ltd, Bury) was compared retrospectively with Phadebact Monoclonal GC coagglutination (Pharmacia Diagnostics, Uppsala, Sweden), cystine trypticase agar sugar utilisation (CTA), and Gonocheck II (J W Turner, Liverpool) enzyme methods for identification of 95 *Neisseria* spp and related species. These had been previously identified using standard methods and included 29 that had given aberrant results. Neisstrip identified correctly all but two, including nine incorrectly identified by Phadebact and 18 erroneously identified using CTA sugars. Results were similar to those obtained with Gonocheck II. After this a prospective study was performed testing 400 oxidase positive isolates derived from clinical samples cultured on gonococcal selective medium. Two organisms, both *Moraxella* spp, were incorrectly identified as *N gonorrhoeae* by the Neisstrip. The superoxol test, when used with either the Phadebact or Neisstrip tests, maintained 100% sensitivity and specificity.

The Neisstrip is a rapid, economic test that is accurate and easy to interpret. It may be used alone or in conjunction with a superoxol test or a coagglutination test, which is relatively accurate but more expensive, and found by some technical staff to be more difficult to interpret.

The identification of *Neisseria gonorrhoeae* by sensitive, precise, and rapid techniques is necessary for the correct diagnosis and appropriate treatment of patients with gonorrhoea. For many years the sugar utilisation tests have been the preferred method, but the advent of rapid methods for detecting antigens or enzyme activity of the bacterium have permitted faster results to be obtained.¹ The falling number of positive cultures for *N gonorrhoeae* in recent years has increased the requirement for tests which are highly reproducible as individual laboratory workers' experience of isolating and identifying *N gonorrhoeae* declines. An increasing number of tests and kits have consequently been appearing on the market, particularly in the United States of America.²

We describe the evaluation of Neisstrip, a 15 minute test which detects the bacterial enzyme β -galactosidase, and prolyl and γ -

glutamyl aminopeptidases. This test was compared with sugar utilisation using cystine trypticase agar (CTA),³ Phadebact Monoclonal GC coagglutination (Pharmacia Diagnostics AB, 75182, Uppsala, Sweden), and the Gonocheck II rapid enzyme detection system (J W Turner Ltd, Liverpool). These kits were evaluated using a culture collection from the Gonococcus Reference Unit which included strains of *N gonorrhoeae* known to give aberrant results in conventional identification tests. We also prospectively evaluated the Neisstrip, Phadebact Monoclonal GC test, CTA test and the superoxol test against unselected isolates of presumptive gonococci in clinical samples from four genitourinary medicine departments, and also strains referred to the Gonococcus Reference Unit from around the United Kingdom.

Methods

Ninety three strains of *Neisseria* spp and related species that had been isolated from clinical samples taken in genitourinary clinics or referred to the Gonococcus Reference Unit were fully identified using standard techniques.³ These isolates would grow on modified New York City medium¹ and were considered to be representative of organisms that could be isolated on gonococcal selective media in clinical laboratories; some, however, had given aberrant results in identification tests. Each of the 93 organisms was tested using the Neisstrip, Gonocheck II, Phadebact Monoclonal GC Test, and sugar utilisation method (CTA sugars).³ The identity of one meningococcus had to be confirmed by the Meningococcal Reference Laboratory, Manchester, England.

For the prospective study clinical samples from the Department of Genitourinary Medicine at Leeds General Infirmary, Leeds, and St Luke's Hospital, Bradford, were cultured on modified Thayer Martin agar (Oxoid Ltd, Basingstoke). Isolates, if found to be oxidase positive Gram negative cocci, were tested using the Phadebact Monoclonal GC Test, the Neisstrip, and, at Leeds, the superoxol test. Any samples for which the results of the first two tests disagreed were further identified using Flynn and Waitkins' serum-free sugar media (Difco, East Molesey) using standard methods.³ Isolates of *N gonorrhoeae* that were referred to the Gonococcus Reference Unit, or isolated in their two genitourinary clinics during the same period

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Table 1 Biochemical characteristics of *Neisseria* capable of growth on gonococcal selective media after Knapp¹

Species	Acid from			β -G	PA	GGA	Superoxol
	Glucose	Maltose	Lactose				
<i>N gonorrhoeae</i>	+	—	—	—	+	—	+
<i>N meningitidis</i>	+	+	—	—	—	+	—
<i>N cinerea</i>	+	—	—	—	+	—	—
<i>B catarrhalis</i>	—	—	—	—	—	—	—
<i>K denitrificans</i>	+	—	—	—	+	—	—

β -G: β -galactosidase, PA: Prolyl or hydroxy prolyl aminopeptidase, GGA: Gamma glutamyl aminopeptidase. *This result may be weak and is not positive in all test systems.

were tested similarly but with Flynn and Waitkins' and CTA tests.

Bacteria that were identified as gonococci using the Neisstrip but not by the other methods were further identified using the API 20NE kit (API Systems, Montalieu-Vervieu, France).

NEISSTRIP COLONY IDENTIFICATION

A single colony was taken from the selective medium and inoculated on to each of the three chemically impregnated filter paper strips that are attached to a plastic strip. Each of the filter papers was moistened with 10 μ l of distilled water, and the strip placed in a Petri dish and incubated at 37°C for 15 minutes. The dish also contained a 2 cm square piece of damp blotting paper to prevent the strip drying out. The development of a blue colour on the β -galactosidase (β -G) strip was taken to indicate the presence of this enzyme, which in turn indicated that this organism could not be *N gonorrhoeae*. If no blue colour appeared a chemical developer was added to the prolyl aminopeptidase (PA) and the γ -glutamyl aminopeptidase (GGA) strips. The formation, within 30 seconds, of a pink colour in the

former and a purple colour in the latter were taken as positive results. Most strains of *N gonorrhoeae* produce PA but not GGA. Other members of the genus *Neisseria* give the combination of results shown in table 1.

PHADEBACT MONOCLONAL GC TEST

This test relies on the presence of the major outer membrane protein (MOMP) protein I on the surface of the bacterium. This protein is only found in strains of *N gonorrhoeae*. It is detected by the coagglutination of a suspension of non-viable *Staphylococcus aureus* cells to which are attached a monoclonal antibody which is specific for the MOMP. A light suspension of the isolate in 0.5 ml of 0.9% (w/v) saline was placed in a test tube in a boiling water bath for five minutes. One drop of each Phadebact reagent was placed on a white card. Each was then mixed with a drop of the boiled extract. The suspensions were gently rocked, and agglutination in one well but not the other within one minute was considered a positive reaction.

GONOCHEK

This system relies, as does Neisstrip, on the differential ability of those Neisseriaceae which will grow on gonococcal selective medium to produce β -G and two aminopeptidases.¹ A heavy suspension of the organism was inoculated from the selective medium into a test tube containing chromogenic substrates for the enzymes. The tube was incubated for 30 minutes at 37°C and the presence of colours in the test tube after this time indicated the presence or absence of enzyme activity.⁴ Yellow was taken to indicate the presence of GGA activity, blue β -G, and red (after the addition of EY-20) PA.

Table 2 Results of comparative identification of 93 bacterial isolates the culture collection of Gonococcal Reference Laboratory

Number of isolates	Neisstrip			CTA sugars			Phadebact monoclonal	Gonochek
	β -G	PA	GGA	Glucose	Maltose	Sucrose		
<i>Neisseria gonorrhoeae</i>								
9	—	+	—	+	—	—	—	Red
4	—	+	—	—	—	—	+	Red
<i>Neisseria meningitidis</i>								
42	—	—	+	+	+	—	—	Yellow
6	—	—	+	+	—	—	—	Yellow
4	—	—	+	—	+	—	—	Yellow
3	—	—	+	—	—	—	—	Yellow
1	—	—	—	+	+	—	—	Yellow
1	—	+	—	+	—	—	—	Red
<i>Neisseria lactamica</i>								
9	+	—	—	+	+	—	—	Blue
1	+	—	—	+	—	—	—	Blue
1	—	+	—	+	+	—	—	Blue
<i>Neisseria cinerea</i>								
2	—	+	—	—	—	—	—	Red
<i>Branhamella catarrhalis</i>								
1	—	+	—	—	—	—	—	Yellow
2	—	—	+	—	—	—	—	Colourless
4	—	—	—	—	—	—	—	Yellow
<i>Moraxella</i> spp.								
2	—	—	+	—	—	—	—	Yellow
1	—	—	—	—	—	—	—	Colourless

β -G: β -galactosidase, PA: prolyl aminopeptidase, GGA: gamma-glutamyl aminopeptidase, CTA: cystine trypticase agar. Gonochek colours: yellow and blue without addition of EY-20, red and colourless after the addition of EY-20. All isolates were capable of growth on gonococcal selective media with exception of *N cinerea*.

Table 3 Results of a prospective study of identification of 400 isolates of *N gonorrhoeae* using Neisstrip, CTA, and Phadebact monoclonal GC identification systems

Number of isolates	Neisstrip			CTA sugars			Phadebact	Identity by API 20NE
	β -G	PA	GGA	Glucose	Maltose	Sucrose		
<i>N gonorrhoeae</i>								
92	—	+	—	+	—	—(24 h)	+	
9	—	+	—	+	—	—(48 h)	+	
2	—	—	—	+	—	—	+	
295	—	+	—	—	—	—	+	
Organisms misidentified as <i>N gonorrhoeae</i>								
2	—	+	—	—	—	—	—	<i>Moraxella</i> spp

β -G: β -galactosidase, PA: prolyl aminopeptidase, GGA: gammaglutamyl aminopeptidase, CTA: cystine trypticase agar.

CTA TEST

Organisms were inoculated on to serum-free cystine trypticase agar (CTA) media containing appropriate sugars, which when degraded during incubation at 37°C for 24–48 hours, produced acid, and thus a change in the colour of the pH indicator present in the media.³ In this study the media were incubated for 48 hours and results compared with those noted after 24 hours. Flynn and Waitkins' test was similarly performed and interpreted.

SUPEROXOL TEST

Using the method of Young *et al.*,⁵ a colony to be tested was rubbed on to a glass slide and a drop of 30% (w/v) hydrogen peroxide added. A positive test was indicated by the production of large numbers of bubbles within two to three seconds.

Results

The four methods used to identify the culture collection isolates all performed well (table 2). The organism that produced PA but not GGA, utilised glucose and maltose, but was Phadebact negative, was identified as a non-groupable *N meningitidis* type 14, subtype p1.15 by the Meningococcal Reference Laboratory at Manchester Public Health Laboratory, which also confirmed our results.

Of the 400 isolates identified in the prospective trial (table 3), all gave consistent results with both the Neisstrip and the Phadebact tests, except for two species of *Moraxella* which had been isolated on gonococcal selective media, and two strains of *N gonorrhoeae*, which failed to give positive PA tests. Of 10 organisms identified using sugar degradation methods during the period of the study, two *N meningitidis* strains were misidentified but gave correct results when using both Phadebact and Neisstrip. The superoxol test gave results consistent with Neisstrip and Phadebact, except that of 29 isolates from the selective media that were not *N gonorrhoeae*, four gave a positive superoxol result (table 4).

Table 4 Superoxol test results on 233 oxidase positive colonies from separate genital swabs cultured on modified Thayer-Martin gonococcal selective agar

Organism identity by Phadebact GC and Neisstrip tests	Superoxol positive	Superoxol negative
<i>Neisseria gonorrhoeae</i>	204	0
Non- <i>N gonorrhoeae</i>	4	25

Discussion

The pressure for accuracy and speed in the identification of *N gonorrhoeae* has led to the development of several commercial kits,² which perform well but add to cost and require refrigeration. In the past the identification of the organism for legal purposes required the production of acid from glucose but not from other sugars. False results do occur and further tests may be required.⁶

The change in the colour of the pH indicator in the medium is slow, difficult to see, and is affected by dissolved carbon dioxide, so this inaccuracy is not surprising.

In the study of the culture collection strains, which included bacteria known to be difficult to identify, none of the organisms was incorrectly identified by both Phadebact and Neisstrip nor by both Phadebact and Gonochek. The reported false positive cross reaction of *N cinerea*, *N lactamica*, *N meningitidis* and *Branhamella catarrhalis* using the pooled monoclonal antibody products in the identification of *N gonorrhoeae*² suggest that further tests may be required to be sure of the identity of a strain. This has not been shown at this time with the Phadebact monoclonal GC test which has been reported as 100% sensitive and specific.⁷ *N cinerea* is exceedingly uncommon in genital specimens but may give a positive sugar utilisation reaction with glucose (and not other sugars). Its identification is therefore difficult. *Neisseria lactamica*, however, is clearly differentiated from *N gonorrhoeae* by both rapid enzyme tests and the CTA test. The same time (15 minutes) is required to use the Neisstrip as the Phadebact test, which may allow them to be carried out concurrently.

The lack of false negative or false positive results when using both Neisstrip and Phadebact monoclonal GC in the culture collection study allowed us to validate the use of further identification methods in the prospective study only if the results of these tests disagreed. The finding that the only two organisms in which this occurred were *Moraxella* spp is not surprising in that these may produce PA and may be found in the upper respiratory tract. To qualify further the occurrence of PA in isolates of the genus *Moraxella* a further three strains were tested and found to be negative.

The advantages of chromogenic substrate tests have been reported.⁸ The ability to give an indication of the identity of an organism that is

not *N gonorrhoeae* is available with Gonocheck II (although the slow production of colour by some strains of *N meningitidis* is a problem⁴), and with a system similar to Neisstrip.⁸ Organisms that did not produce any colour change were considered to be *Branhamella catarrhalis* in these studies, but it is clear that this pattern can be produced by other organisms,⁸ including the two *Moraxella* spp in this study. We feel it would be unwise to identify *B catarrhalis* using Neisstrip or Gonocheck II, and their use to identify *N meningitidis* should only be in conjunction with further tests. They would be useful, however, to help identify aberrant strains of meningococci that are maltose negative.⁵ *N subflava* biovar *perflava* or *Kingella denitrificans* do not occur commonly in pharyngeal samples, but their ability to produce PA may cause them to be misidentified by both Neisstrip or Gonocheck II as *N gonorrhoeae* from gonococcal selective media. Hence, the use of either alone to test colonies from the pharynx may prove inadequate. Similarly, the cross reactions of other monoclonal antibody based tests with pharyngeal Neisseriaceae² may suggest that these too should not be used alone in identification of gonococci from the pharynx. Pharmacia's Monoclonal GC Test, however, is not directly comparable with these other tests.²

The superoxol test requires experience to differentiate positive and negative results but gave results consistent with Neisstrip and with Phadebact Monoclonal GC in 98% of organisms tested in the prospective study. It is rapid, and cheap, but shows some positive results with other Neisseriaceae, particularly *N lactamica*, *N meningitidis*, and *B catarrhalis*.⁵ Non-gonococcal isolates from gonococcal selective

medium, when tested by the superoxol test and Neisstrip, gave a false positive rate which we estimate to be less than 0.1%. Use of these two tests would require that if only one was positive then the result should be checked by other methods.

We conclude that Neisstrip is a convenient, rapid system for the identification of *N gonorrhoeae* from selective media for the isolation of gonococci, and that it may be of use in the identification of other pathogenic Neisseriaceae. When used alone in the circumstances that we describe it has a sensitivity of 99%, a specificity of 95% giving a positive predictive value of 99%. In combination with the superoxol test it is exceedingly accurate.

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