A rapid slide coagglutination test—an alternative to the fluorescent antibody test for the identification of *Neisseria gonorrhoeae*

S SHANKER, DA DALEY, AND TC SORRELL*

From the Bacteriology Department, Institute of Clinical Pathology and Medical Research, Westmead, and the *Department of Medicine, Infectious Disease Medicine Unit, Westmead Centre, Westmead, New South Wales, Australia

SUMMARY The Phadebact^(R) Gonococcus Test, a slide coagglutination test, was compared with the Difco fluorescent antibody test for the identification of *Neisseria gonorrhoeae* isolated from 18- to 24-hour primary plates. A total of 316 morphologically characteristic, oxidase-positive, Gram-negative diplococci were tested. Altogether 298 isolates were identified definitively as *N. gonorrhoeae* by a rapid carbohydrate utilisation test; 287 of the 298 isolates of *N. gonorrhoeae* were identified by the coagglutination test, a sensitivity of 96%. The sensitivity of the fluorescent antibody test was 85% (254 of 298 isolates). False-positive results due to cross-reactions with non-gonococcal *Neisseria* were uncommon (1 of 18 non-gonococcal isolates in the coagglutination test, a specificity of 94%; 2 of 18 in the fluorescent antibody test, a specificity of 88%). None of 14 other contaminant organisms seen frequently on primary isolation media gave positive reactions. Interpretation of the coagglutination test proved to be difficult initially. Thirty-two (10%) coagglutination tests had to be repeated; 3 of the 32 (1% of the total isolates tested) remained uninterpretable.

The use of staphylococcal protein A coagglutination tests for the serological identification of various bacteria is well documented.¹⁻¹⁰ A commercial kit based on the principle of coagglutination has been produced by Pharmacia Diagnostics AB, Uppsala, Sweden, for the identification of *Neisseria gonorrhoeae*. This kit was recently introduced to the Australian market.

At the beginning of our study there had been only two evaluations of this kit, one in Sweden⁹ and the other on a prototype of the kit in the United Kingdom.¹⁰ Both studies utilised pure subcultures of confirmed *N. gonorrhoeae* but the results of the two evaluations were not in agreement. We undertook to assess the sensitivity and specificity of the kit available in Australia and to determine if the test could be carried out on primary isolates. This would allow a rapid identification of *N. gonorrhoeae* within 18 to 24 hours of the specimen being taken.

The coagglutination test was compared with a fluorescent antibody test currently used in our laboratory for the routine screening of gonococcal-

like organisms isolated from primary plates.

Material and methods

Specimens were obtained from patients attending the Venereal Diseases Clinic in Sydney. Over a six-month period 1000 specimens were obtained. These included throat, urethral, and cervical swabs from females, and throat, urethral, and rectal swabs from males.

Swabs were plated directly on to Modified New York City (MNYC) medium¹¹ at the clinic and incubated overnight at 36°C in BBL jars with CO₂ Gaspaks^(R). The jars were then transported to the laboratory 18 to 24 hours later. After this period of incubation colonies of *N. gonorrhoeae* measured about 1 mm in diameter.

An oxidase test and a Gram stain were performed on colonies morphologically similar to *N. gonorrhoeae*. Oxidase-positive, Gram-negative diplococci were tested with a fluorescent antibody test and the coagglutination test. Results of these tests were read independently before identification of the organism by the rapid carbohydrate utilisation test described by Young *et al.*¹²

FLUORESCENT ANTIBODY TEST

The fluorescent antibody test was performed using Difco reagents. A culture of *N. gonorrhoeae* was used as a positive control and cultures of *Neisseria meningitidis* and *Enterobacter cloacae* were used as negative controls for each batch of tests.

COAGGLUTINATION TEST

The reagents were supplied as a commercially available test kit, Phadebact^(R) Gonococcus Test, provided by Pharmacia (South Seas) Pty Ltd.

Reagents

The test package contained lyophilised 'gonococcal reagent', 'control reagent', and 'buffer' in powder-form. The gonococcal reagent consisted of anti-gonococcal antiserum raised in rabbits and bound to inactivated staphylococci. The control reagent consisted of gammaglobulin from non-immunised rabbits bound to inactivated staphylococci. The reconstituted buffer powder provided a buffer solution of 7.4. This solution was used to reconstitute, wash, and resuspend the gonococcal and control reagents. The reconstituted reagents had a shelf-life of one month at 5°C. The reagents were brought to room temperature before the test.

Procedure

Drops of each of the gonococcal and control reagents were placed approximately 3 cm apart on a clean, dry glass slide. Four or five suspected gonococcal colonies were picked from the primary MNYC plate, and two thin smears were made adjacent to the drops of reagents. Each smear was mixed well with the corresponding drop of reagent. The slide was then held close to a source of warmth, for example, a desk lamp, rocked for 2 minutes, and viewed under transillumination against a dark background.

Interpretation

A positive reaction was identified by the development of fine powdery particles—coagglutination—in the drop containing gonococcal reagent. The drop of control reagent remained as a thin milky suspension. A negative reaction was one in which there was no coagglutination in the drop of gonococcal reagent. A non-interpretable result was one in which coagglutination was evident in both gonococcal and control drops. Where results were not interpretable, the following two methods were used:

(1) A drop of a 0.1% solution of trypsin was applied to the dried smears before the addition of the test reagents. This modification of the method was suggested by Menck,⁹ who claimed it reduced considerably the number of non-interpretable results.

(2) As recommended by the manufacturer, a 0.5 ml aqueous suspension of several colonies was heated in a water bath at 80°C for 20 minutes and cooled to room temperature. Drops of the aqueous suspension were used for retesting.

The specificity of the coagglutination test was evaluated by testing other *Neisseria* spp and non-*Neisseria* commonly encountered on primary isolation plates.

RAPID CARBOHYDRATE UTILISATION TEST

The test, based on work done by Kellog and Turner¹³ and Young *et al.*¹² measures the action of preformed enzymes on specific substrates: glucose, sucrose, fructose, lactose, and maltose. As the test requires a heavy inoculum, all rapid carbohydrate utilisation tests were carried out on pure, 24-hour subcultures of suspect colonies that were oxidase-positive, Gram-negative diplococci.

Reagents

Stock carbohydrate solutions. 10% (w/v) stock solutions of glucose, sucrose, fructose, lactose, and maltose* were prepared in distilled water, dispensed in 4-ml amounts, and stored at -20°C.

Phosphate buffered salt solution. A solution of pH 7.1-7.15, consisting of 40 ml 0.1 M K₂ HPO₄, 12 ml 0.1 M KH₂PO₄, 100 ml 8% (w/v) KCl, 10 ml 1% (w/v) aqueous phenol red, and 838 ml sterile distilled water was prepared; 300-μl aliquots were dispensed into bijou bottles and stored at -20°C.

Of the carbohydrate solutions 20-μl aliquots were dispensed into the wells of BBL Minitek^(R) trays; 100 μl of the phosphate buffered salt solution were then added to each of the wells. The wells were labelled and sealed, and the trays were stored at -20°C.

Procedure

The respective solutions were thawed immediately before use, and a heavy emulsion of the inoculum was prepared using the 300 μl buffer solution; 20 μl of this emulsion were then inoculated into each carbohydrate-containing well in the Minitek^(R) tray. The trays were incubated in a 37°C water bath for 3 hours. A pH change, indicated by a colour change from red to yellow in the wells, showed the presence of preformed enzyme for the specific substrate.

Results

Of the 1000 plates screened, 316 isolates were identified presumptively as *N. gonorrhoeae* by their

*Maltose—extra pure (BDH Chemicals Ltd, UK).

characteristic morphology, positive oxidase reaction, and Gram stain. A total of 298 isolates (94%) were confirmed as *N. gonorrhoeae* by our rapid carbohydrate utilisation test; 18 isolates were identified as non-gonococcal *Neisseria* species (Table 1). Of 298 proven gonococcal isolates, 286 were identified by the coagglutination test and 252 by the fluorescent antibody test, a sensitivity of 96% and 85%, respectively. These differences were significant ($P < 0.0005$, χ^2 test). The specificity of the test (percentage of non-gonococcal *Neisseria* yielding a negative result) was 94% for the coagglutination test (17 of 18 isolates) and 88% for the fluorescent antibody test (16 of 18 isolates ($P > 0.5$, χ^2 test)). No false-positive results were obtained with non-*Neisseria* species which commonly contaminate primary isolation plates (Table 2). False-negative results were obtained in 9/298 coagglutination tests compared with 46/298 fluorescent antibody tests ($P < 0.0005$, χ^2 test).

The results of 32 (10%) coagglutination tests were not interpretable on initial testing. Use of the alternative method recommended by the manufacturer led to interpretation of all but three of the tests. These three isolates were later identified as *N. gonorrhoeae*. The modification of Menck⁹ failed to discriminate results not interpretable by coagglutination testing.

Discussion

Our data indicate that the coagglutination test is a practicable alternative to the fluorescent antibody test in the rapid screening of suspect gonococcal colonies from primary isolation plates. It is more sensitive than the fluorescent antibody test, detecting 96% of isolates identified as *N. gonorrhoeae*, compared to 85% using the fluorescent antibody test. The specificity of the coagglutination test is similar to that of the fluorescent antibody test, 94% of non-gonococcal *Neisseria* yielding a negative result compared to 88% with the fluorescent antibody test. Barnham and Glynn¹⁰ were critical of the lack of specificity of the coagglutination test but they did not use an alternative method for testing organisms giving inconclusive results. Of 35 non-gonococcal *Neisseria* tested by them, 12 were coagglutination positive and 12 were inconclusive. Menck⁹ tested 16 non-gonococcal *Neisseria*. After trypsinisation, 12 were negative and four were inconclusive. We tested 18 non-gonococcal *Neisseria*; 17 were negative and one was positive, a *N. meningitidis* isolated from a throat swab. Pharyngeal colonisation by *N. meningitidis* has been reported in approximately 10% of patients attending a venereal diseases clinic,¹⁵ and in up to 25% of normal males.¹⁶ The organism has also been iso-

Table 1 Fluorescent antibody (FA), coagglutination (CoA), and rapid carbohydrate utilisation test (RCUT) results on 316 Gram-negative diplococci that were oxidase positive

Origin of specimen	No. of specimens	FA		CoA			RCUT	
		Positive	Negative	Positive	Negative	Not interpretable	Positive*	Negative
Urogenital	236	198	38	227	8	1	236	0
Rectal	60	52	8	57	2	1	59	1
Throat	20	4	16	3	16	1	3	17
Total	316	254	62	287	26	3	298	18

*Carbohydrate reactions consistent with *N. gonorrhoeae*.¹⁴

Table 2 Fluorescent antibody (FA) and coagglutination (CoA) results for other organisms found on primary isolation plates

Organism	No. tested	FA		CoA	
		Positive	Negative	Positive	Negative
<i>Neisseria meningitidis</i>	15	2	13	1	14
<i>Neisseria catarrhalis</i>	2	0	2	0	2
<i>Neisseria pharyngis</i>	1	0	1	0	1
<i>Candida</i> spp.	3	0	3	0	3
<i>Escherichia coli</i>	3	0	3	0	3
<i>Klebsiella</i> spp.	2	0	2	0	2
<i>Staphylococcus albus</i>	2	0	2	0	2
<i>Streptococcus faecalis</i>	2	0	2	0	2
<i>Streptococcus viridans</i>	2	0	2	0	2
Mixed cultures of <i>N. gonorrhoeae</i> and normal flora	10	10	0	10	0

isolated from genitourinary and anal specimens.¹⁷⁻¹⁹ As the carriage of *N. meningitidis* and *N. gonorrhoeae* carries different therapeutic implications, it is important to differentiate these two species of *Neisseria*. In our study, other non-gonococcal species of *Neisseria* did not cross-react with *N. gonorrhoeae*.

The coagglutination test costs no more per test than the fluorescent antibody test and it is less time-consuming. The rapidity of the test (less than 2 minutes per test for most isolates) and the fact that expensive laboratory equipment is not required make it attractive for smaller laboratories. The shelf-life of the reconstituted coagglutination reagents, however, is one month when stored at 5°C, and smaller laboratories would process fewer gonococcal isolates during that period.

Clearly, the coagglutination test cannot replace carbohydrate tests for definitive identification of *N. gonorrhoeae* (13/298 (4%) gonococcal isolates defined by rapid carbohydrate utilisation test were identified incorrectly by coagglutination testing). It is sufficiently sensitive and specific to replace the fluorescent antibody test in the rapid screening of suspect colonies from primary isolation plates.

We thank Pharmacia (South Seas) Pty Ltd for supplying us with the Phadebact^(R) Gonococcus Test kits. Thanks are also extended to Dr R Munro for her encouragement and assistance during the course of this study.

References

- Kronvall G. A rapid slide-agglutination method for typing pneumococci by means of specific antibody adsorbed to protein A-containing staphylococci. *J Med Microbiol* 1973;**6**:187-290.
- Christensen P, Hahlmeter G, Johsson S, Kronvall G. New method for the serological grouping of streptococci with specific antibodies adsorbed to protein A-containing staphylococci. *Infect Immun* 1973;**7**:881-5.
- Jublin J, Winbald S. Serotyping of *Mycobacteria* by a new technique using antibody globulin adsorbed to staphylococcal protein A. *Acta Pathol Microbiol Scand* (B) 1976;**81**:179-80.
- Danielson D, Kronvall G. Slide agglutination method for the serological identification of *Neisseria gonorrhoeae* with antigonococcal antibodies adsorbed to protein A-containing staphylococci. *Appl Microbiol* 1974;**27**:368-74.
- Edwards EA, Larson GL. New method of grouping beta-haemolytic streptococci directly on sheep blood agar by coagglutination of specifically sensitised protein A-containing staphylococci. *Appl Microbiol* 1974;**28**:972-6.
- Hahn G, Nyberg I. Identification of streptococcal groups A, B, C and D by slide agglutination of antibody-sensitised protein A-containing staphylococci. *J Clin Microbiol* 1976;**4**:99-101.
- Arvilommi H. Grouping of beta-haemolytic streptococci by using coagglutination, precipitation or bacitracin sensitivity. *Acta Pathol Microbiol Scand* (B) 1976;**84**:79-84.
- Maxted WR, Efstratiou A, Parker MT. Agglutination grouping of streptococci (Letter). *Lancet* 1976;**2**:692-3.
- Menck H. Identification of *Neisseria gonorrhoeae* in cultures from tonsillopharyngeal specimens by means of a slide agglutination test (Phadebact® Gonococcus Test). *Acta Pathol Microbiol Scand* (B) 1976;**84**:139-44.
- Barnham M, Glynn AA. Identification of clinical isolates of *Neisseria gonorrhoeae* by a coagglutination test. *J Clin Pathol* 1978;**31**:189-93.
- Young H. Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. *Br J Vener Dis* 1978;**54**:36-40.
- Young H, Paterson IC, McDonald DR. Rapid carbohydrate utilization test for the identification of *Neisseria gonorrhoeae*. *Br J Vener Dis* 1976;**52**:172-5.
- Kellog DS, Turner EM. Rapid fermentation confirmation of *Neisseria gonorrhoeae*. *Appl Microbiol* 1973;**25**:550-2.
- Cowan ST. *Cowan and Steele's Manual for the Identification of Medical Bacteria*. 2nd ed. Cambridge University Press, 1974.
- Noble RC, Cooper RM, Miller BR. Pharyngeal colonisation by *Neisseria gonorrhoeae* in black and white patients attending a venereal disease clinic. *Br J Vener Dis* 1979;**55**:14-9.
- Greenfield S, Sheehe PR, Feldman HA. Meningococcal carriage in a population of 'normal' families. *J infect Dis* 1971;**123**:67-73.
- Willmott FE. Meningococcal salpingitis. *Br J Vener Dis* 1976;**52**:182-3.
- Miller MA, Millikin P, Griffin PS. *Neisseria meningitidis* urethritis. A case report. *JAMA* 1979;**242**:1656-7.
- Givan KF, Thomas BW, Johnston AG. Isolation of *Neisseria meningitidis* from the urethra, cervix, and anal canal. Further observations. *Br J Vener Dis* 1977;**53**:109-12.

Requests for reprints to: Mr S Shanker, Bacteriology Department, Institute of Clinical Pathology and Medical Research, Old Windsor Road, Westmead 2145, NSW, Australia.