Evaluation of a fluorescent monoclonal antibody reagent for identification of cultured *Neisseria gonorrhoeae*

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Summary. We evaluated a new fluorescent monoclonal antibody reagent for confirmation of identity of *Neisseria gonorrhoeae*. The reagent correctly identified all 161 fresh clinical isolates of *N. gonorrhoeae*, which included 11 penicillinase producing strains (PPNG). The reagent also correctly identified 21 stored PPNG strains. No cross reactions were seen with 58 fresh clinical isolates of *N. meningitidis*, 12 stored strains of *N. lactamica*, or with strains of *Gardnerella vaginalis*, lactobacilli, *Candida* spp., *Staphylococcus epidermidis* or Enterobacteriaceae. Some cross reaction was noted with strains of *S. aureus*, probably related to cell-wall protein A. However, this reagent was highly sensitive and specific for use against oxidase positive, gram-negative cocci isolated in London.

Introduction

Identification of *Neisseria gonorrhoeae* in a clinical laboratory must be rapid and accurate, and may be of medico-legal as well as clinical significance. In the United Kingdom it is most commonly achieved by conventional carbohydrate-utilisation tests which require pure cultures and at least overnight incubation. Alternative methods include rapid carbohydrate-degradation tests based on the principle described by Kellogg and Turner (1973), enzymic tests with chromogenic substrates (Janda *et al.*, 1985), or immunological methods (Young and Reid, 1984). All these rapid techniques produce a result within 4 h of isolation of the presumptive neisseria.

Two types of immunological method are currently available, coagglutination and direct fluorescent staining of the organism. The sensitivity of coagglutination by a monoclonal reagent at 95–100% is better than that of the polyclonal antibody system originally used (Barnham and Glynn, 1978; Philip *et al.*, 1984; Young and Reid, 1984). However, commercially available fluorescent antibody reagents, usually containing polyclonal antibody, have suffered from a lack of both sensitivity and specificity (Freundlich *et al.*, 1982). We have evaluated a new fluorescent-antibody (FA) reagent

(Syva Co., Palo Alto, CA, USA) containing monoclonal IgG antibodies.

Material and methods

Strains

A total of 161 isolates of *N. gonorrhoeae* (including 11 PPNG strains) and 58 isolates of *N. meningitidis* was obtained from primary cultures from patients attending the Praed Street Clinic, London. The source of the isolates is shown in table I. A further 21 isolates of PPNG and 12 isolates of *N. lactamica* from our collection of cultures stored in liquid nitrogen were also tested.

A range of bacterial isolates (numbers tested in brackets) including *Gardnerella vaginalis* (10), lactobacilli (1), *Staphylococcus epidermidis* (5), *S. aureus* (4), *Candida* spp. (3), *Escherichia coli* (5), *Klebsiella oxytoca* (1), *K. pneumoniae* (1), *Proteus vulgaris* (1), *Enterobacter agglomerans* (1) and *Acinetobacter* sp. (1) was tested to evaluate the specificity of the reagent.

Culture

All specimens from patients attending the clinic were inoculated directly onto neisseria isolation medium containing GC agar base (BBL) 36g/L, IsoVitalex (BBL) 1%, vancomycin 3 µg/ml, colistin 100 units/ml, trimethoprim 5 µg/ml, and amphotericin 1.5 µg/ml. After inoculation, plates were incubated at 36°C in an atmosphere of CO₂ 7% in air until transported to the laboratory, where they were re-incubated for up to 48 h. Stored isolates of

Table I. Source of primary isolates of *Neisseria* spp.

Organism	Number of isolates tested from									
	Total	Male patients						Female patients		
		Heterosexual			Homosexual					
		U	P		U	R	P	U	C	P
<i>N. gonorrhoeae</i> (non-PPNG)	150	69	0	11	8	0	17	45	0	
<i>N. gonorrhoeae</i> (PPNG)	11	7	0	2	0	0	0	2	0	
<i>N. meningitidis</i>	58	0	11	0	2	36	0	0	9	

U = urethra, P = pharynx, R = rectum, C = cervix.

PPNG and *N. lactamica* were cultured on the above medium without the addition of antibiotics.

Isolates of *G. vaginalis* and lactobacilli were grown on 5% human-blood agar, staphylococci on 5% horse-blood agar, *Candida* spp. on Sabouraud's agar and the remaining isolates on MacConkey agar.

Identification

Neisseria spp. were identified as oxidase-positive, gram-negative cocci. *N. gonorrhoeae* was distinguished from other neisseriae by its ability to utilise only glucose. Carbohydrate-utilisation tests were performed on serum-free medium containing glucose, maltose or sucrose poured into petri dishes (Flynn and Waitkins, 1972). Penicillinase-producing strains were detected with a chromogenic cephalosporin (nitrocefin, Oxoid) (O'Callaghan *et al.*, 1972). *N. lactamica* was distinguished from *N. meningitidis* by its ability to split ortho-nitrophenyl-galactoside as previously described (Ison *et al.*, 1984).

G. vaginalis isolates were identified as oxidase- and catalase- negative, gram-variable bacilli that were β -haemolytic on human- but not horse-blood agar. Staphylococci were identified as catalase-positive gram-positive cocci and distinguished by coagulase and DNAase activity. All gram-negative bacilli isolated on MacConkey agar were identified by API20E strips (API Laboratory Products Ltd).

Immunofluorescence technique

A thin smear of each type of colony was prepared by lightly touching five colonies and emulsifying in a 5- μ l drop of distilled water on a glass slide. The smears were allowed to dry completely in air and then gently heat fixed. The fluorescent-antibody reagent (Microtrak; Syva Co.) 30 μ l, was added to each test smear, and to smears of known positive and negative control organisms. The slides were then incubated for 15 min at 37°C in a well-humidified chamber to prevent drying which may lead to incorrect results. After incubation, excess reagent was removed by shaking and the slides rinsed for 5–10 s with a gentle stream of distilled water. The smears were then

thoroughly dried in air and mounted in the solution provided. When possible, slides were read immediately or stored at 2–8°C in the dark and read within 24 h.

The degree of fluorescence was determined with a Laborlux microscope (Leitz Instruments) and a 100 $\times$ oil-immersion objective. Each smear was graded: 0 = no fluorescence; 1+ = all cocci were green and fluoresced at a low intensity; 2+ = all cocci fluoresced, with clearly visible rims, medium intensity; 3+ = all cocci fluoresced brightly with very clear rims; 4+ = all cocci fluoresced very brightly.

Results

From 219 primary cultures containing oxidase-positive gram-negative cocci, 161 strains of *N. gonorrhoeae* (including 11 PPNG) and 58 strains of *N. meningitidis* were correctly identified by the FA reagent. The fluorescent reactions of these isolates together with the 21 stored strains of *N. gonorrhoeae* and 11 of *N. lactamica* are shown in table II. All 182 strains of *N. gonorrhoeae* gave positive fluores-

Table II. Results of fluorescent-antibody reagent tests with various *Neisseria* spp.

Organism	Total number tested	Number that gave fluorescence intensity				
		0	1+	2+	3+	4+
Primary isolates						
<i>N. gonorrhoeae</i> (non-PPNG)	150	0	6	31	49	64
<i>N. gonorrhoeae</i> (PPNG)	11	0	0	4	2	5
<i>N. meningitidis</i>	58	58	0	0	0	0
Stock isolates						
<i>N. gonorrhoeae</i> (PPNG)	21	0	2	8	5	6
<i>N. lactamica</i>	12	12	0	0	0	0

cent reactions, all but eight of which were $\geq 2+$ intensity. The age of the culture did not affect this intensity. None of the strains of *N. meningitidis* or *N. lactamica* reacted with the fluorescent-antibody reagent.

Of all the other species tested only three strains of *S. aureus* showed any fluorescence. This was seen in only a minority of cocci, and these staphylococci did not resemble typical gonococci morphologically. No attempt was made to block this reaction with serum of any kind as this commercial reagent is intended for use solely with oxidase-positive gram-negative cocci.

Discussion

The FA reagent correctly identified all the isolates of *N. gonorrhoeae* from primary cultures plates. The test was simple and quick to perform and gave easily readable results. It was used successfully on colonies without subculture and was not affected by the viability or age of the culture. The isolates of PPNG tested in this study all gave a positive result. Immunologically-based gonococcal reagents have been reported to fail to identify some isolates of PPNG (Waitkins and Anderson, 1982; Philip *et al.*, 1984). In our experience, some strains of PPNG

are slow to utilise carbohydrates, particularly after only one or two subcultures. This FA reagent offers a useful alternative which would enable quick identification and reporting of a penicillin-resistant isolate.

We did not detect any cross-reactivity with other species of *Neisseria* tested. In our laboratory *N. meningitidis* and *N. lactamica*, but not other neisseriae are commonly isolated from pharyngeal cultures. When used against oxidase-positive, gram-negative cocci isolated on our neisseria selective medium, the reagent was 100% specific.

Spurious staining was only observed with isolates of *S. aureus*. This was probably due to protein A on these strains which binds IgG non-specifically, resulting in positive fluorescence. This problem is encountered with any reagent that uses IgG antibody and it does not present a practical problem in a clinical laboratory where only oxidase-positive, gram-negative cocci are tested.

This immunofluorescence test is particularly useful because it requires neither viable organisms nor the overnight incubation of subcultures which is frequently necessary for carbohydrate-utilisation tests of any type. Its sensitivity, specificity and ease of use make it an attractive alternative to other rapid tests.

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