

Failure of the fluorescent antibody reaction to identify penicillinase-producing gonococci

SHEENA A WAITKINS,* R DAWN ANDERSON

From the Regional Public Health Laboratory, Fazakerley Hospital, Lower Lane, Liverpool L9 7AL

SUMMARY The fluorescent antibody test is now widely used to confirm the identity of *Neisseria gonorrhoeae* but may fail to identify penicillinase-producing strains (PPNG). This problem arises when conjugates are used that incorporate only gonococci that are not penicillinase-producers. We have shown that conjugates prepared from mixtures of PPNG and non-penicillinase producing gonococci give good fluorescent reactions.

This difference in the reactions of PPNG strains is clearly related to their penicillinase-producing abilities, further study of the antigenic relation between penicillinase production and the antigenic structure of *N gonorrhoeae* is evidently required.

Since penicillinase-producing *Neisseria gonorrhoeae* (PPNG) first appeared in Liverpool in February 1976^{1,2} they have been identified throughout the world.³⁻⁵ The existence of such strains represents an epidemiological problem particularly in developing countries where expensive treatment such as spectinomycin is not readily available. Whenever such strains are isolated it is clearly necessary to confirm their identity. If many strains from patients and contacts are to be examined, biochemical identification including sugar fermentation tests may be too time-consuming for the clinical laboratory to perform routinely. It has become common practice to substitute the fluorescent antibody technique (FAT)^{6,7} for these biochemical methods in an effort to identify rapidly suspect organisms. However, this may lead to false-positive identification of *Neisseria gonorrhoeae* particularly with other *Neisseria*—for example, *N lactamica* and *N meningitidis*, both of which may exhibit non-specific immunofluorescence with FITC-labelled antigonococcal conjugate.⁷

Nevertheless, provided that these limitations are understood, organisms that resemble Gram-negative diplococci and are oxidase- and FAT-positive isolated from patients who have admitted venereal contact are almost always identified as *N gonorrhoeae* in routine laboratories.

We have found that PPNG may show weak or frankly negative fluorescence when tested by FAT and may not be identified as *N gonorrhoeae* if this

technique is the only means of identification in the laboratory.

Material and methods

ORGANISMS

Strains of *N gonorrhoeae* were isolated from patients attending sexually transmitted disease clinics in Liverpool and Birkenhead, Merseyside, during 1978, 1979 and 1980. Penicillinase-producing *N gonorrhoeae* previously isolated from 1976 which had been preserved in liquid nitrogen were examined (strains 76/16353; 76/325777; 76/38401; 76/78980 and AP 672), as were recent freshly isolated strains (78/5414; 78/72767 and 78/74163) (Table 1). Non-penicillinase-producing gonococci were preserved in liquid nitrogen from early 1978 (Table 2).

MEDIA

The routine specimens were inoculated on to Thayer Martin medium⁸ containing vancomycin 2 µg/ml, colistin 12.5 µg/ml, nystatin 12.5 units/ml, and trimethoprim 5 µg/ml.⁹ The organisms were identified by colony form, Gram-stain, a positive oxidase reaction and the fermentation of glucose but not sucrose, maltose nor lactose. Fermentation reactions were carried out using a modified carbohydrate medium consisting of Difco GC medium base with 1% sugar concentrations.¹⁰ FITC-labelled anti-gonococcal conjugate was prepared by the method of Lind.^{7,11} Two different batches of anti-gonococcal conjugate were made. The first was prepared in 1975, and was a laboratory-prepared

Accepted for publication 13 July 1981

*Present address: Public Health Laboratory, Glan Clwyd Hospital, Bodelwyddan, near Rhyl, Clwyd LL18 5UJ.

Table 1 *Penicillinase-production by Neisseria gonorrhoeae and their variation in fluorescent antibody reaction*

Organism	Penicillin MIC (units/ml)	Commercially prepared conjugate	Laboratory prepared conjugate (LPC)	LPC incorporating antibody to PPNG
Penicillinase-producing <i>Neisseria gonorrhoeae</i>				
78/5414	> 100	±	+++	+++
78/7688	> 100	-	+++	+++
78/72767	> 100	±	±	+++
78/74163	> 100	±	+++	+++
76/16353	> 100	-	+++	+++
76/325777	> 100	-	+++	+++
76/38401	> 100	±	+++	+++
76/78980	> 100	±	+++	+++
AP 672	> 100	±	+++	+++
Negative control (<i>N meningitidis</i>)		±	±	±

PPNG = penicillinase-producing *Neisseria gonorrhoeae*.
 ± → +++ = degrees of positive reaction.
 ± = equivocal result recorded as negative.
 - = negative.

Table 2 *Penicillinase-production by Neisseria gonorrhoeae and variation in fluorescent antibody reaction*

Organism	Penicillin MIC (units/ml)	Commercially prepared conjugate	Laboratory prepared conjugate (LPC)	LPC incorporating antibody to PPNG
Non-penicillinase-producing <i>Neisseria gonorrhoeae</i>				
78/68350	0.5	±	+++	+++
78/68352	0.06	±	+++	+++
78/68660	0.125	±	+++	+++
78/69490	0.03	±	+++	+++
78/70463	0.008	±	+++	+++
78/4358	0.5	±	+++	+++
78/15291	0.06	±	+++	+++
78/15713	0.015	±	+++	+++
78/15715	0.125	±	+++	+++
78/16149	0.008	±	+++	+++
78/16151	0.008	±	+++	+++
Negative control (<i>N meningitidis</i>)		±	±	±

PPNG = penicillinase-producing *Neisseria gonorrhoeae*.
 ± → +++ = degrees of positive reaction.
 ± = equivocal result recorded as negative.
 - = negative.

conjugate (LPC) which did not contain PPNG. The second batch, prepared in 1978 (LPCPPNG) contained three of those organisms selected from PPNG in Table 1 (78/5414; 78/74163; and AP 672). Commercially prepared FITC-labelled antigenococcal conjugate was obtained from Difco Laboratories Ltd, using recommended dilution of 1 part conjugate to 3 parts distilled water. Fluorescent antibody tests were performed on all gonococci using the method described by Lind.^{7 11}

Briefly, the suspect colony was mixed with distilled water to form a thin film on a multispot PTFE coated slide (Hendley Essex) and then dried and fixed gently with heat. One drop of either Difco or laboratory-prepared conjugate was allowed to react with the gonococcus at 37°C for 15 to 20 min in a moist box. After this time the film was washed twice in phosphate buffer, the final washing lasting at least

5 min. The slide was then allowed to dry under a normal bench lamp, mounted in glycerol phosphate buffer and viewed under oil immersion on a Zeiss standard microscope equipped for fluorescence microscopy. The degree of fluorescence was recorded in values from 0 to +++. The value +++ corresponds to brilliant, apple-green fluorescence, whereas ++ was less brilliant. A positive reaction was characterised by the typical morphology of the organism and by the degree of fluorescence (+++), a reading of ++ indicated a cautious positive identification.

ANTIBIOTIC SENSITIVITIES

All strains of *N gonorrhoeae* isolated were tested for sensitivity to penicillin by a 3-disc method using discs (Mast) containing respectively 0.03, 0.25 and 1 units of benzyl penicillin.¹² The diameter of each

zone of inhibition is converted into minimum inhibition concentration (MIC) value from a standard curve prepared from tube dilution determinations.

Penicillinase activity of the gonococcus was tested by smearing a loopful of culture on to a filter paper previously saturated with chromogenic cephalosporin 500 µg/ml (Glazo Ltd).¹³ The colour of the reagent changes from yellow to deep red within five minutes in the presence of the enzyme β-lactamase. For comparison a stock strain of *Escherichia coli* known to produce penicillinase was used as a positive control.

Results

During routine testing of all isolates by FAT, it was noted that one PPNG 78/5414, appeared to give consistently diminished fluorescence with the commercially prepared conjugate. On further testing with our own LPC it still performed weakly when compared with the reactions given by non-penicillinase producing gonococci using the FAT method.

Table 2 illustrates our findings when non-penicillinase producing organisms were examined by FAT. Regardless of their MIC to penicillin, there was little variation when commercial or our own LPC was used. The slight differences noted, although perhaps sufficient to mislead inexperienced workers, should not interfere with normal identification, provided that the commercial conjugate is used within one month after reconstitution and kept in the dark at 4°C.

These differences, however, became much more marked when PPNG were examined. Table 1 shows our results when a number of penicillinase-producing gonococci both freshly isolated and preserved in liquid nitrogen were tested against both commercial and LPC conjugate. In general, penicillinase-producing gonococci performed poorly with the commercial preparation. Indeed, strains 78/7688 and 78/16353 were considered to be negative while 78/72767 and 76/38401 gave equivocal results. When compared with our own laboratory prepared conjugate (LPC), the results obtained were slightly better, and only 78/72767 remained negative.

In view of the importance placed on positive FAT identification by most laboratories, we decided to repeat the FAT with a FITC-labelled antigenococcal conjugate from a freshly prepared gonococcal pool which included three strains of PPNG (78/5414; 78/72767 and AP 672). There was a conspicuous increase in fluorescence when the new conjugate was used with PPNG (Table 1, column 3). These organisms gave good positive reactions; even those that were previously either negative or doubtful showed strong fluorescence. There was no change in the strongly positive reactions of non-penicillinase

producing organisms (Table 2). Similar improvement in fluorescent reactions was seen when PPNG isolates from 1979 and 1980 were tested. This variation in fluorescent reaction was independent of conjugate dilution used.

Discussion

The reliable identification of *Neisseria gonorrhoeae* is clearly important in relation to the diagnosis, treatment and epidemiological control of gonorrhoea and sometimes for medicolegal purposes.

The fluorescent antibody technique (FAT) is frequently used as a rapid confirmatory test. It is recognised that the FAT conjugate used must be highly specific for *N. gonorrhoeae* and must not fluoresce with other *Neisseria* spp such as *meningitidis* or *lactamica*.

We have drawn attention to another problem in the use of this test in that commercially-prepared conjugates may give false-negative results with penicillinase-producing gonococci (PPNG). In our hands, fluorescence with commercial conjugate or a conjugate prepared in our laboratory (LPC) was markedly reduced or absent. In the case of both the commercial conjugate and the LPC, the antigen used to raise specific antibody did not contain PPNG. However, when PPNG were included in the antigen pool used in the preparation of the conjugate, strongly positive fluorescent reactions were obtained with both non-penicillinase producing gonococci and PPNG. It is clear that the failure of PPNG to react in the FAT with conjugate prepared from non-penicillinase producing gonococci is related to penicillinase production. There are evidently antigenic differences between PPNG and non-penicillinase producing strains which require further investigation.

We would like to thank Dr GC Turner, Director, Liverpool Public Health Laboratory, for help in preparing this manuscript, and Mrs G Stringfellow for typing it.

References

- 1 Percival A, Rowlands J, Corkhill JE, *et al.* Penicillinase-producing gonococci in Liverpool. *Lancet* 1976;ii:1379-82.
- 2 Turner GC, Ratcliffe JG, Anderson RD. Penicillinase-producing *Neisseria gonorrhoeae*. *Lancet* 1976;ii:793.
- 3 Asford WA, Golash RG, Hemming VG. Penicillinase-producing *Neisseria gonorrhoeae*. *Lancet* 1976;ii:657-8.
- 4 Piot P, Roberts M, Niname G. B-lactamase production in commensal Neisseriaceae. *Lancet* 1979;i:619.
- 5 Centre for Disease Control. Epidemiologic holes and reports: follow up on antibiotic resistant *Neisseria gonorrhoeae*. *Morbidity and Mortality Weekly Report* 1977;26:29-30.

- ⁶ Daniellson D. The demonstration of *N gonorrhoeae* with the aid of fluorescent antibodies. *Acta Derm Venereol (Stockh)* 1963;**43**:451-64.
- ⁷ Lind I. Identification of *Neisseria gonorrhoeae* by means of fluorescent antibody technique. *Acta Pathol Microbiol Scand* 1967;**70**:613-29.
- ⁸ Thayer JD, Martin JE. A selective media for the cultivation of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. *Public Health Rep* 1964;**79**:49-57.
- ⁹ Seth AD. Use of trimethoprim to prevent overgrowth by *Proteus* in the cultivation of *N gonorrhoeae*. *Br J Vener Dis* 1970;**46**:201-2.
- ¹⁰ Flynn J, Waitkins SA. A serum-free medium for testing fermentation reactions in *Neisseria gonorrhoeae*. *J Clin Pathol* 1972;**25**:525-7.
- ¹¹ Lind I. Methodologic aspects of routine procedures for identification of *Neisseria gonorrhoeae* by immunofluorescence. *Ann NY Acad Sci* 1975;**254**:400-6.
- ¹² Wilkinson AE, Turner GC, Rhycroft JA. Laboratory diagnosis of venereal disease. In: Willis AT, Collins CH, eds. London: PHLs Monograph No 1, 1972:35-9.
- ¹³ O'Callaghan CH, Morris A, Kirby SM, Shingler AH. Novel method for detection of β -lactamase by using a chromogenic cephalosporin substrate. *Antimicrob Agents Chemother* 1972;**1**:283-8.

Requests for reprints to: Dr Sheena A Waitkins, Public Health Laboratory, Glan Clwyd Hospital, Bodelwyddan, Near Rhyl, Clwyd LL18 5UJ.