

# Serotyping *Neisseria gonorrhoeae*: a report of the Fourth International Workshop

M J Gill

An International Workshop was held at Datchet, UK in May 1990 to discuss the serotyping of *Neisseria gonorrhoeae*. The aim of the workshop was to bring together workers from around the world to exchange information and ideas on current applications of serotyping gonococci. The workshop also aimed to identify problems that have arisen with serotyping, and to facilitate their resolution. Three sessions were held: on the application of serotyping to epidemiology and to antibiotic resistance, and on the methodology of serotyping.

## Background

The major outer membrane and porin of *N gonorrhoeae*, Protein I (PI) is used to serotype this organism. PI is found in two structural subclasses.<sup>1</sup> These have been designated IA and IB. Any isolate of *N gonorrhoeae* will usually express only one subclass of PI. However, within each subclass there is antigenic variation which allows further subdivision into serovars. The two panels of monoclonal antibodies to these epitopes which have been most widely used are: the Genetic Systems (GS) panel from Syva, Palo Alto, USA<sup>2</sup> and a panel from Pharmacia (Ph), Uppsala, Sweden.<sup>3</sup> A third panel also exists (GonoType<sup>TM</sup>; New Horizons Diagnostics, Columbia, USA<sup>4</sup>). The patterns of coagglutination reactions of an isolate with one of the panels of antibody is used to assign a serovar.

Both numerical and descriptive nomenclatures have been applied to the GS-panel.<sup>5,6</sup> When the Knapp GS system is used<sup>7</sup> these nomenclatures are interchangeable.<sup>7</sup> Serovars determined by the Ph panel are always designated by descriptive nomenclature. While designation of an isolate as having a IA or IB serogroup is the same with the GS- and Ph-panels, subdivision into serovars is not comparable with these two panels. The use of different antibody panels for serotyping is therefore confusing.

## Applications of serotyping

### Epidemiology

Since the last International workshop,<sup>8</sup> serotyping has been used extensively worldwide. It has been used to monitor the incidence and prevalence of isolates of gonococci within and between populations. Many groups also auxotype isolates and combine this characteristic with the serovar to produce auxotype/serovar classes (A/S classes).<sup>9</sup> Using A/S classes increases discrimination between strains. This may be extremely useful in forensic work or in contact-tracing. However, overdiscrimination may cause confusion when attempting correlations with other phenotypic traits such as antibiotic resistance or site tropism.

There may be marked differences in serovars found in close geographical areas. Young *et al*<sup>10</sup> (data also presented at workshop) have studied four adjacent areas in Scotland. When data from these areas were combined, 11 IA serovars were present. However, the IA-4 serovar was found only in Edinburgh (Lothian) in homosexual men, and the IA-21 serovar was found only in Glasgow. IB-17 serovar strains that do not react with the Pharmacia confirmation reagent (Phadebact GC OMNI Test<sup>TM</sup>) have been found only in Glasgow. In Bath IB-2 serovar strains are twice as common as in nearby Bristol (A Jephcott, data presented at workshop). The IA-2 serovar is more common in Bristol than in Bath.

While geographical variation in serovars may occur, some associations with serovars are worldwide in distribution. For example, isolates from homosexuals are often associated with IB serovars. In Scotland such isolates tend to be IB-2, in Canada they are IB-16, -3, -4 or -10, in Australia they are IB-4 or -7, and in London they are IB-2 (H Young, JR Dillon, J Forsyth, data presented at workshop<sup>11</sup>). These findings may represent the effect of strain transmission within homosexual populations. However, it is possible that the IB structural subtype of the gonococcal porin also gives such strains a biological advantage. Gonococcal isolates from the hydrophobic environment of the rectum are known to be less permeable to hydrophobic molecules than those from the urethra.<sup>12</sup>

Epidemiological studies assume that the serovar,

---

Department of Medical Microbiology, St Mary's Hospital Medical School, Norfolk Place, London W2 1PG, UK  
M J Gill

---

and also the auxotype, are stable characteristics of a given strain. There are few published data demonstrating any contribution to antigenic drift in PI serovar of in vivo immunological selective pressure in cases of reinfection. Because of the lack of an animal model for gonorrhoea, and the difficulties of human volunteer work, any evidence must come from clinical studies. Such studies require large stable populations in which the introduction of strains from outside can readily be identified. Changes in the incidence of particular serovars might then represent antigenic drift. A population such as Hong Kong would not be appropriate. Here, although the strain population is very heterogeneous, there appear to be persistent and transient strains. The persistent strains appear most commonly in the large number of highly active and mobile prostitutes. However, it is not clear whether these strains are established or constantly being introduced by international travellers (S Eglestone, data presented at workshop). Several other studies<sup>9-15</sup> have also demonstrated, within defined populations, a stable background of strains with particular serovars or A/S classes. New serovars (A/S classes) appear sporadically to become established or to disappear. For example, in Avon (A Jephcott, data presented at workshop) the A/S classes AHU/IA-2, non-requiring (NR)/IB-3 and pro<sup>-</sup>arg<sup>-</sup>/IB-4 were present throughout 1989. Arg<sup>-</sup>/IB-3 and his<sup>-</sup>/IB-3 strains appeared later and became established in this population. Over a 14 month period, Plummer *et al*<sup>16</sup> demonstrated that prostitutes being infected with a strain of specific serovar had a reduced risk of reinfection with the same serovar. This was associated with an increase in frequency of IB-1 and IB-5 strains, and a decrease in IA-4 and IB-3 strains. However, in this study and others, changes due to strain importation into the population, asymptomatic carriage or antibiotic selection cannot be discounted.

Studies of the concordance of phenotypic characteristics of isolates from different sites in the same patient, or from partner-pairs might also be expected to give an indication of serovar stability in vivo (EW Hook, data presented at workshop<sup>9,13</sup>). In isolate-pairs with identical auxotype and antibiogram, most discrepancies in serovar are due to single epitope differences. This could be accounted for by technical problems (see below). Other discrepancies may be due to acquisition of strains from outside the partnership or mixed infections in a single patient.

Most clinical isolates of gonococci are thought to have one gene for PI (*por*), with the two subtypes of PI as alleles.<sup>17,18</sup> Hence the vast majority are either of serogroup IA or IB. Isolates have, however, been found that express a mixture of IA and IB epitopes<sup>19</sup> (CA Ison, personal communication). Hence genetic events in vivo can generate antigenic diversity in PI.

There are few in vitro data relating to serovar stability. At St Mary's Hospital Medical School we have subjected many strains to multiple passages on conventional media. We have never found any change in serovar (unpublished data). While there is currently no indication of immunological selection of gonococci in vitro, some evidence that this might occur has been demonstrated in *Neisseria meningitidis*. In vitro growth of meningococci in the presence of appropriate antibody and complement, has resulted in mutants deficient in class 1 and class 3 outer membrane proteins.<sup>20</sup> These proteins are structural and functional analogues of gonococcal PI. Current evidence indicates that within a strain, PI serovar is reasonably stable. This contrasts with other components of the gonococcal cell wall. Protein II (PII) demonstrates marked antigenic variation even during the course of infection in a single individual.<sup>21,22</sup> Pili also undergo phase and antigenic variation.

#### *Antibiotic resistance*

Serotyping has been used widely in the study of gonococcal antibiotic resistance. IB serovars continue to be associated with chromosomally-mediated resistant *N gonorrhoeae* (CMRNG). Such strains have been found in Bahrain,<sup>23</sup> Canada, Zimbabwe, Mexico and London (JR Dillon, P Mason, E Calderon and C Conde-Glez, CSF Easmon, data presented at workshop).

Reduced susceptibility to 4-fluoroquinolones has been found in the UK and Australia. The Gonococcus Reference Unit, Bristol has received 20 such isolates since 1988 (A Turner, data presented at workshop). Thirteen of these isolates did not react with the GS-panel. However, they typed as "hybrid" IA/IB (Bj/AvBx) with the Ph panel. Similar isolates have also been reported in Scotland.<sup>19</sup> Such isolates have epidemiological links to Spain. In London, 23 isolates with reduced ciprofloxacin susceptibility have been described. The predominant A/S classes in these isolates are non-requiring (NR)/IB-6 and arg<sup>-</sup>/IB-6 (I Phillips and C Warren, data presented at workshop). Since 1984, 30 isolates with reduced susceptibility to ciprofloxacin have been found in Australia (J Tapsall, data presented at workshop). These strains generally possessed higher levels of intrinsic resistance to other antibiotics. That these isolates are a heterogeneous group is indicated by the presence of 20 A/S classes. However, once again IB serovars predominate. It is thought that most of these isolates are imported from the Philippines.

Extensive serotyping of penicillinase-producing *N gonorrhoeae* (PPNG) has been performed. Isolates of PPNG containing the 4.4 MDa penicillinase plasmid are more commonly of IB serovars than those with the 3.2 MDa penicillinase plasmid, which are of IA serovars. This association has been found in Germany, Mexico and the United Kingdom (P Kohl,

E Calderon and C Conde-Glez, I Phillips, C Warren and CSF Easmon, data presented at workshop). However, most PPNG currently found in the Netherlands contain the 3.2 MDa plasmid, with IB-1 as the predominant serovar (MDessens-Kroon, data presented at workshop).

PPNG containing the 3.05 MDa penicillinase plasmid isolated in the USA are all of the non-requiring (NR)/IB-1 A/S class. In vitro attempts to transfer this plasmid have failed to date (S Sarafian, data presented at workshop). This may be attributed to a non-functional 3.05 or 24.5 MDa conjugative plasmid. Canadian strains containing the 3.05 MDa plasmid are of several A/S classes (JR Dillon, data presented at workshop): NR/IB-2 (65%), NR/IB-1 (12%), NR/IB-3 (11%), NR/IB-6 (6%), NR/IA-6 (4%) and NR/IB-4 (2%).

Serotyping has been used to study the outbreak of high-level resistance to tetracycline in *Neisseria gonorrhoeae* (TRNG) in the Netherlands<sup>24</sup> (B van Klingeren, data presented at workshop). The TRNG first isolated in 1985 were all NR/IB-4 PPNG, containing the 3.2 MDa penicillinase plasmid. No TRNG were isolated in the Netherlands in 1986. However, there has been an annual increase in TRNG in the three years 1987-9. Most of these TRNG are also 3.2 MDa plasmid-containing PPNG. Three A/S classes predominate among the TRNG over this period: NR/IB-6, pro<sup>-</sup>/IA-3 and NR/IB-3. TRNG are most common in the Hague/Rotterdam area of the Netherlands. Preliminary figures for TRNG isolated during the first three months of 1990 indicate that this epidemic may be diminishing.

### Methodology of serotyping

The standard GS-panel of 12 antibodies, together with the Knapp nomenclature,<sup>5</sup> is the most widely used system for designating serovars. Twelve groups represented at the workshop use this system. Three groups have access to the Ph-panel. The consensus at the workshop was that the GS-panel and Knapp nomenclature should therefore be used universally.

### Technical reproducibility

Several factors may affect the reproducibility of coagglutination for serotyping. These include the intrinsic avidity of an antibody for its epitope, the activity of various batches of antibody, and reading of coagglutination reactions. The presence and strength of coagglutination reactions depends on the area of the slide over which they are performed; a stronger reaction is obtained when reagents are spread over a larger area. It is therefore essential that the slides are clean and free from grease before use. To minimise these variables, the GS-panel is provided to research workers via distributors. Distributors are responsible for the training of new users, reagent preparation

and help with problems that arise. A recent world-wide quality control survey of 11 centres that use the GS-panel has demonstrated generally good reproducibility between different centres and with different batches of antibodies (CA Ison, data presented at workshop). This study did, however, find variation between centres in the reactivity of antibodies 2D6, 2G2, and to a lesser extent 6D9, to standard strains. Antibodies 2D6 and 6D9 tend to precipitate during coagglutination reactions. They have also been found to be difficult antibodies from which to prepare coagglutination reagents. Purification of antibody and standardisation of the concentration used may resolve these problems.

In an attempt to improve reproducibility and ease of reading coagglutination reactions, a system incorporating methylene blue into the serotyping reagents has been devised (M Carballo and JR Dillon, data presented at workshop). In this system coagglutination reactions were performed in a microtitre plate. Concordance of results obtained from the standard coagglutination protocol with results from the modified system was generally good. However, the reproducibility of the modified system was reduced. This may be due to performing coagglutinations over the small area available in microtitre plates, rather than the inclusion of methylene blue in the system.

### Non-typable strains and subtyping within serovars

Isolates that are non-typable by the standard GS-panel have been reported by several groups (JR Dillon, CA Ison, S Sarafian; communication at workshop). These isolates probably represent less than 1% of all the strains on which serotyping has been performed. Many "non-typable" isolates react with antibody 2H7. To date, all strains found non-typable with the GS-panel have been found to type with the Ph panel. Isolates which do not type with the GS-panel using the standard protocol should be further assessed using antibody 2H7 and the Ph-panel. Central collection and collation of these data will enable assessment of the extent of this problem and whether new antibodies should be added to the panel. It may also provide information on genetic drift in PI. This is essential to ensure that serological detection and identification systems for gonococci remain effective.

Subtyping within the IB-1 serovar has been attempted with antibody 3B10(j) (CA Ison, data presented at workshop). However, no differentiation of strains in relation to site of isolation, or penicillin susceptibility has yet been found.

### New methods

Evaluation of serotyping by enzyme-linked immunosorbent assay (ELISA) is also being performed (P Kohl; M Carballo and JR Dillon, data presented at workshop). Unlike the coagglutination serotyping

reactions which use boiled cells to expose PI epitopes, the Kohl ELISA uses an unboiled suspension of cells. Discrepancies between this ELISA and standard coagglutination were found mainly with GS-panel antibodies 5D1, 2D6 and 2HI. The Carballo/Dillon ELISA uses boiled cells as antigen. Control strains are used to calculate the "cut-off" values for each epitope. Non-specific reactions are few, but antibody 4G5 is not detected by this ELISA. Whether these methods will find wide-spread use in improving reproducibility or with non-typable strains is not yet known.

In the future techniques other than serotyping or auxotyping may be found to be of use in the identification of biologically significant categories within existing serovars. These may include isoenzyme typing and restriction fragment length polymorphism (RFLP) analysis. Such techniques may also contribute to the grouping together of certain serovars. This has already been suggested for IA-1 and IA-2, for IB-2 and IB-16 strains of the PAOU auxotype, and for serovars IB-5 and IB-7.<sup>8</sup>

#### Conclusion and recommendations

Gonococcal serotyping has proved useful in the study of clinical epidemiology, antibiotic resistance and pathogenesis. At this workshop a consensus was reached on the following points. Standardisation of serotyping is important. Since the GS-panel with the Knapp nomenclature is the most widespread system for serotyping, it should be adopted wherever possible. At present there appears to be no need to alter the standard GS-panel of antibodies.<sup>5</sup> As many laboratories as possible should take part in the quality control (QC) assessment of serotyping. Some laboratories have offered to contribute to the cost of the QC scheme.

Isolates that are non-typable with the GS-panel should be referred for typing with the Ph-panel and other antibodies. H Young and A Moyes have agreed to assess these isolates with the Ph-panel and CA Ison, JR Dillon and S Sarafian have agreed to do this using 2H7 and other GS antibodies.

Centralised collation and widespread dissemination of QC results and further studies of "non-typable" isolates, will give workers confidence in the veracity of results they obtain. It will also help to monitor antigenic drift.

In future, other techniques such as a PI-ELISA, isoenzyme typing or RFLP analysis may be of use in assessing non-typable isolates or grouping serovars into biologically significant categories. Progress in the problem areas identified at this fourth International Workshop will need to be reviewed at a future workshop.

#### Workshop participants

KM Bindayna, Arabian Gulf University, Bahrain; E

Calderon and CJ Conde-Glez, Instituto Nacional de Salud Publica, Cuernavaca, Mexico; M Carballo and JR Dillon, Laboratory Centre for Disease Control, Ottawa, Canada; J Clay, The General Hospital, Birmingham, England; K Culshaw, Bayer UK Ltd, Newbury, England; M Dessens-Kroon, B van Klingeren and M Verheul, RIVM, Bilthoven, The Netherlands; S Egglestone, Hong-Kong Polytechnic; JRL Forsyth, University of Melbourne, Australia; K Gough, A Jephcott and A Turner, Gonococcus Reference Unit, Bristol, England; M Haggman, Orebro Medical Center Hospital, Sweden; EW Hook and TM Neumann, Johns Hopkins Hospital School of Medicine, Baltimore, USA; I Kamionek and P Kohl, Universitäts-Hautklinik, Heidelberg, Germany; P Mason, University of Zimbabwe Medical School, Harare; A Moyes and H Young, The Medical School, Edinburgh, Scotland; I Phillips and C Warren, St Thomas' Hospital, London, England; TO Rod, University of Oslo, Norway; S Sarafian, Center for Disease Control, Atlanta, USA; J Tapsall, The Prince of Wales Hospital, Randwick, Australia; E van Dyck, Institute of Tropical Medicine, Antwerp, Belgium; N Branley, CSF Easmon, MJ Gill, CA Ison and G Onwuchekwa, St Mary's Hospital Medical School, London, England.

The organisers (Dr CA Ison and Prof. CSF Easmon, Dept. of Medical Microbiology, St Mary's Hospital Medical School) and participants of this workshop thank its sponsors: The Wellcome Trust, Upjohn Ltd, Bayer UK Ltd, and the Syva Company of Palo Alto. I thank all workshop participants, in particular Dr CA Ison and Prof. CSF Easmon, for allowing me to report their work and for their constructive criticism during preparation of this manuscript. MJG is supported by a Wellcome Trust Medical Graduate Fellowship.

Address for correspondence: Dr MJ Gill.

- Judd RC. Protein I: structure, function and genetics. *Clin Microbiol Rev* 1989;2 **Suppl**:S41-S48.
- Tam MR, Buchanan TM, Sandstrom EG, et al. Serological classification of *Neisseria gonorrhoeae* with monoclonal antibodies. *Infect Immun* 1982;36:1042-53.
- Sandstrom E, Lindell P, Harfast B, Blomberg F, Ryden A-C, Bygdeman S. Evaluation of a new set of *Neisseria gonorrhoeae* serogroup W-specific monoclonal antibodies for serovar determination. In: Schoolnik GK, Brooks GF, Falkow S, Frasch CE, Knapp JS, McCutchan JA, Morse SA, eds. *The Pathogenic Neisseriae*. Washington DC: American Society for Microbiology, 1985:26-30.
- Kohl PK, Olsen DA, Buchanan TM. Monoclonal antibodies to protein I for serotyping of *Neisseria gonorrhoeae*: correlation of serotype with bactericidal antibody. *Zentralbl Bakteriol Mikrobiol Hyg [A]*. 1989;270:517-26.
- Knapp JS, Tam MR, Nowinski RC, Holmes KK, Sandstrom EG. Serological classification of *Neisseria gonorrhoeae* with use

- of monoclonal antibodies to gonococcal outer membrane protein I. *J Infect Dis* 1984;150:44-8.
- 6 Bygdeman SM, Gillenius E-C, Sandstrom EG. Comparison of two different sets of monoclonal antibodies for the serological classification of *Neisseria gonorrhoeae*. In: Schoolnik GK, Brooks GF, Falkow S, Frasch CE, Knapp JS, McCutchan JA, Morse SA, eds. *The Pathogenic Neisseriae*. Washington DC: American Society for Microbiology, 1985;31-6.
  - 7 Knapp JS, Bygdeman S, Sandstrom E, Holmes KK. Nomenclature for the serological classification of *Neisseria gonorrhoeae*. In: Schoolnik GK, Brooks GF, Falkow S, Frasch CE, Knapp JS, McCutchan JA, Morse SA, eds. *The Pathogenic Neisseriae*. Washington DC: American Society for Microbiology, 1985;4-5.
  - 8 Kohl PK, Ison CA, Danielsson D, Knapp JS, Petzoldt D. Current status of serotyping of *Neisseria gonorrhoeae*. *Eur J Epidemiol* 1990;6:91-5.
  - 9 Knapp JS, Sandstrom EG, Holmes KK. Overview of epidemiological and clinical applications of auxotype/serovar classification of *Neisseria gonorrhoeae*. In: Schoolnik GK, Brooks GF, Falkow S, Frasch CE, Knapp JS, McCutchan JA, Morse SA, eds. *The Pathogenic Neisseriae*. Washington DC: American Society for Microbiology, 1985;6-12.
  - 10 Young H, Moyes A, Robertson DHH, et al. Gonococcal infection within Scotland: antigenic heterogeneity and antibiotic susceptibility of infecting strains. *Eur J Epidemiol* 1990;6:1-8.
  - 11 Woodford N, Bindayna KM, Easmon CSF, Ison CA. Associations between serotype and susceptibility to antibiotics of *Neisseria gonorrhoeae*. *Genitourin Med* 1989;65:86-91.
  - 12 Morse SA, Lysko PG, McFarland L, et al. Gonococcal strains from homosexual men have outer membranes with reduced permeability to hydrophobic molecules. *Infect Immun* 1982;37:432-8.
  - 13 Knapp JS, Holmes KK, Bonin P, Hook EW. Epidemiology of gonorrhoea: distribution and temporal changes in auxotype/serovar classes of *Neisseria gonorrhoeae*. *Sex Trans Dis* 1987;14:26-32.
  - 14 Ison CA, Spencer RC. Epidemiology of *Neisseria gonorrhoeae* in Sheffield. (Abstract). *J Med Microbiol* 1990;31:vii.
  - 15 Bindayna KM, Ison CA. Sampling methods for monitoring changes in gonococcal populations. *Epidemiol Infect* 1989;103:203-9.
  - 16 Plummer FA, Simonsen JN, Chubb H, et al. Epidemiologic evidence for the development of serovar-specific immunity after gonococcal infections. *J Clin Invest* 1989;83:1472-6.
  - 17 Gotschlich EC, Seiff ME, Blake MS, Koomey M. Porin protein of *Neisseria gonorrhoeae*: cloning and gene structure. *Proc Nat Acad Sci USA* 1987;84:8135-9.
  - 18 Carbonetti NH, Sparling PF. Molecular cloning and characterisation of the structural gene for protein I, the major outer membrane protein of *Neisseria gonorrhoeae*. *Proc Nat Acad Sci USA* 1987;84:9084-8.
  - 19 Young H, Moyes A, Tait IB, McCartney AC, Gallacher G. Nontypable quinolone resistant gonococci. *Lancet* 1990;335:604.
  - 20 Tommassen J, Vermeij P, Struyve M, Benz R, Poolman JT. Isolation of *Neisseria meningitidis* mutants deficient in class 1 (porA) and class 3 (porB) outer membrane proteins. *Infect Immun* 1990;58:1355-9.
  - 21 Swanson J, Koomey JM, Boslego J. Gonococcal pilin and PII variation *in vivo* (Abstract). Proceedings, *Sixth International Pathogenic Neisseria Conference*, Pine Mountain, Georgia, USA. 1987;181.
  - 22 Saunders JR. Modulating bacterial virulence. *Nature* 1989;338:622-3.
  - 23 Bindayna KM. The use of typing systems to monitor antibiotic resistance in *Neisseria gonorrhoeae*. PhD Thesis, University of London. 1989.
  - 24 van Klingeren B, Dessens-Kroon M, Verheuve M. Increased tetracycline resistance in gonococci in the Netherlands. *Lancet* 1989;ii:1278.

Accepted for publication 9 October 1990.