Evaluation of *PorB* Variable Region Typing of *Neisseria gonorrhoeae* Using PCR-ELISA in Samples Collected From Men Who Have Sex With Men

A.E. Ling,¹ M.C. Bash,² F. Lynn,² N.A. Lister,³ P. Zhu,⁴ S.M. Garland,^{1,5}
C.K. Fairley,^{3,6} and S.N. Tabrizi^{1,5*}

¹Department of Microbiology and Infectious Diseases, The Royal Women's Hospital, Melbourne, Australia

²Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland

³Melbourne Sexual Health Centre, Melbourne, Australia

⁴Crete MicroTech, Inc., Potomac, Maryland

⁵Department of Obstetrics and Gynaecology, University of Melbourne, Melbourne, Australia

⁶School of Population Health, University of Melbourne, Melbourne, Australia

A polymerase chain reaction (PCR)-based enzyme-linked immunosorbent assay (ELISA) methodology was developed to characterize *Neisseria gonorrhoeae* *porB* gene variable regions (VR); the methodology was evaluated in comparison to *porB* VR typing by checkerboard hybridization. Clinical noncultured samples from 35 men who have sex with men (MSM), positive by nucleic amplification assays for *N. gonorrhoeae*, were typed using a panel of 40 oligonucleotide probes to *porB* VRs and compared to checkerboard hybridization. Complete concordance was observed between the two methods at PIB VRs 1, 3, and 7. At the more degenerate VRs 5 and 6, PCR ELISA resulted in obtaining more typeable VRs than checkerboard

hybridization due to single nucleotide mismatches. By PCR ELISA, two predominant PIB *porB* types were identified in 58% of the samples and the remaining 16 samples had one of six other *porB* types. Both PCR ELISA and checkerboard hybridization methods of *porB* VR typing allowed characterization of *N. gonorrhoeae* from noncultured clinical samples including throat and rectal swabs and discriminated *N. gonorrhoeae* from *N. meningitidis* present in some of the samples. PCR ELISA is a rapid, relatively inexpensive and alternative molecular typing method for *N. gonorrhoeae*, suitable for use in conjunction with molecular diagnostic tests. J. Clin. Lab. Anal. 21:237–243, 2007. ©

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INTRODUCTION

Neisseria gonorrhoeae remains a common cause of sexually transmitted infection (STI) in developed countries. Over the past decade the number of STIs, including infections with *N. gonorrhoeae*, has increased in Australia, particularly among men who have sex with men (MSM) (1,2). MSM who become infected with *N. gonorrhoeae* may be asymptomatic and at risk of developing serious chronic conditions and transmitting infection to sexual partners if infection is not detected or is untreated. In order to develop rational public health strategies, the dynamics of the spread of the disease needs to be well understood. Rapid and accurate methods to characterize the prevalent *N. gonorrhoeae* strains are necessary for this purpose.

Monoclonal antibodies (MAbs) directed against the outer membrane protein, *PorB* have traditionally been employed for serovar typing of *N. gonorrhoeae* infections (3,4). However, the use of antigenic epitopes of *PorB* in serotyping is limited due to problems with reproducibility and availability of MAbs (5). Antigenic and other

The experiments comply with the current laws of Australia and the United States.

*Correspondence to: S.N. Tabrizi, The Royal Women's Hospital, 132 Grattan Street, Carlton, Victoria 3053, Australia.

E-mail: sepehr.Tabrizi@rch.org.au

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traditional methods of typing also require culture isolation of the bacterium that relies on stringent transport conditions necessary for the delivery of viable specimens.

More recently, nucleic acid amplification tests (NAATs) have been employed to directly identify infectious microorganisms by polymerase chain reaction (PCR) amplification of nucleic acid sequences specific to that pathogen. NAAT diagnostic methods often provide a higher level of sensitivity and specificity for pathogens than more conventional techniques. Several molecular methods for typing *N. gonorrhoeae* have also been developed and include typing based on deoxyribonucleic acid (DNA) restriction analysis such as pulsed-field gel electrophoresis or PCR-restriction fragment length polymorphism (RFLP) *opa* typing (6–10). Other molecular typing methods utilize DNA sequencing of specific genes such as *porB*, *thpB*, or *gyrA* (8,11–14). Multilocus sequence typing examines polymorphic sites in multiple housekeeping genes to distinguish strains (15).

N. gonorrhoeae possess one of two single-copy *porB* gene alleles, *porB1a* or *porB1b*, that express *PorB1a* (PIA) or *PorB1b* (PIB) proteins, respectively. *PorB* is of interest as a typing target because it is the target of traditional serologic typing antibodies and has been shown to be important in host–pathogen interactions (3,16–21). The protein has eight predicted surface-exposed loops and several of these loops may be associated with antigenic regions of the protein (22). The corresponding gene regions that encode these loops display a number of possible variations that account for differences in antigenic types of *N. gonorrhoeae* and can be used to genetically type strains (5,23). *porB* VR typing by checkerboard hybridization utilizes oligonucleotide probes to the regions of the gene that have greatest sequence diversity. This method is accurate and discriminatory, utilizes synthetic probes, can type direct clinical specimens, and can identify coinfection with more than one gonococcal strain (24–27). Because of the potential advantages of a hybridization method for use with direct clinical specimens and the relative ease of enzyme-linked immunosorbent assay (ELISA) based methods, we compared PCR ELISA to the established checkerboard hybridization method using a selection of PIA and PIB control strains and a collection of noncultured clinical samples from MSM that were PCR-positive for *N. gonorrhoeae*.

MATERIALS AND METHODS

N. gonorrhoeae Isolates and Specimens

The sequence confirmed positive and negative control strains for PIA VR typing (252, 177007, PI83, DGI27,

and 192) and PIB VR typing (MS11, 3744, 9299, and 2432) were used for this study (24). Specimens, representing first-pass urine, self-collected rectal swabs, and clinician-collected throat swabs, were collected from 521 MSM tested at male-only saunas in Melbourne, Australia, over a 12-month period between 2002 and 2003 to study the prevalence of STIs (2). Extracted, stored DNA from *N. gonorrhoeae*-positive samples were typed in this study. The project was approved by the Victorian Department of Human Services Human Research Ethics Committee. Each sample had been tested for *N. gonorrhoeae* by PCR targeting the *cppB* gene (2,28) and the *opa* gene (29). A total of 43 samples (two urine samples, 22 rectal swabs, and 19 throat swabs) were collected from 32 MSM PCR-positive for *N. gonorrhoeae* (2). Three MSM had *N. gonorrhoeae* detected in both throat and rectal swabs. One male had *N. gonorrhoeae* detected from repeat positive throat and rectal samples and another male had *N. gonorrhoeae* detected in repeat positive urine and rectal samples.

DNA Extraction

Control strain genomic DNA was isolated using a Wizard genomic DNA purification kit (Promega Corp., Madison, WI). Genomic DNA from each clinical specimen was isolated as samples were collected using the automated MagNA Pure LC (Roche Diagnostics, Indianapolis, IN) with the associated DNA Isolation Kit I protocol and stored at -70°C and batch tested in this study.

PCR Amplification of *porB*

Amplification of *porB* was performed on DNA from six PIA and three PIB control strains as described (24). A nested PCR was performed for amplification of the *porB* gene from the 43 clinical specimens as previously described with the exception of using 2.5 U of AmpliTaq Gold DNA polymerase (Roche Molecular Systems, Branchburg, NJ) (27). Second-round *porB* amplification reactions were similar to the first reaction except for using 1 μL of the first-round PCR product as the template, 50 μM digoxigenin (DIG)-2'-deoxyuridine 5'-triphosphate (dUTP) for the PCR ELISA samples, and 1 μM each of primers PIB.Fpr (5'-ATTGCCCT-GACTTTGGCAGCCCTTCCT) and PIB.Rpr (5'-TTGCAACCAGCCGGCAGAAACCAAGGC) (24). PCR amplicon size and concentration were determined by electrophoresis in 1.5% agarose gel, ethidium bromide staining, and the GelVue UV Transilluminator and GeneSnap gel documentation and Analysis system (Syngene, Cambridge, UK).

Checkerboard Hybridization

Checkerboard hybridization was performed as described previously by hybridization of VR sequence-specific biotin-labeled probes to PCR-amplified *porB* applied to a nylon membrane (24). A panel of 40 probes corresponding to loops 1, 2, 3, 6, and 7 for the PIA porin, and 1, 3, 5, 6, and 7 for the PIB porin were employed to characterize VR types of the *porB* gene (26). Hybridization temperature was 59°C for all probes. Checkerboard hybridization was performed on two or three independent PCR amplifications of DNA purified from clinical samples. In the checkerboard assay all samples were tested against all probes and the definition of a positive VR signal was based on visual comparison to the signal intensity of control strains with known *porB* sequences.

PCR-ELISA

A 5-μL aliquot of DIG-labeled amplification product from each sample was denatured by incubating with 0.4 M NaOH for 10 min. Hybridization solution (Roche Diagnostics, Indianapolis, IN) containing 50 ng/mL of 5'-biotinylated oligonucleotide probe were added into streptavidin-coated microtiter plate wells (Roche Diagnostics). Denatured PCR products were added and hybridized for 2 hr, at 5° below melting temperature (T_m), 55°C for all probes except for probes PIB1-2, PIB1-3, PIB3-1 and PIB3-2 that were hybridized at 50°C and PIB1-1, PIB5-4, PIB5-7 and PIB6-6 that were hybridized at 60°C. Wells were washed six times with PCR ELISA wash buffer (Roche Diagnostics) in an automated microplate washer. PCR products that bound to the immobilized probes were detected by adding peroxidase-conjugated anti-DIG antibody (Roche Diagnostics) diluted in conjugate buffer (Roche Diagnostics) and incubated at 37°C for 30 min. The wells were washed and incubated with colorimetric peroxidase substrate 2,2-azino-di-(3-ethylbenzthiazoline sulfonic acid) ammonium salt solution (ABTS) (Roche Diagnostics) at 37°C for 30 min. The absorbency of hybridized products was detected at 410 nm in a MRX plate reader (MUREX, Chicago, IL).

For each VR probe tested, positive and negative bacterial control strains were chosen based on the presence or absence of the most similar VR typing sequence, respectively, as determined by checkerboard hybridization and the *porB* sequences for each strain (26). Based on evaluations of other PCR ELISA assays (data not shown), optical density (OD) values equal or greater than 2.5 times the OD value obtained for the negative control for that VR, were considered positive for that VR. OD values less than 2.5 times the OD value obtained for the negative control for that VR were considered negative for the VR probe.

Sequencing of the *porB* Gene

The sequence of *porB* was obtained by direct sequencing of second-round unlabelled, amplified PCR products. The purified PCR product was sequenced using the DTCS Quick Start Kit (Beckman Coulter, Fullerton, CA) according to the manufacturer's instructions. The sequence primers used were PIB.F and PIB.R, GCseq.F471 (5'-TTGAAAGGCGGCTTCGG-3'), GCseq.R502 (5'-CGCCGGTGTTCAGG-3'), and GCseq.F851 (5'-CTGCAAGTTCACCGTTTGG-3'). DNA sequence data were obtained using a Beckman Coulter CEQ 8000 automated DNA sequencer (Beckman Coulter, Fullerton, CA) according to the manufacturer's instructions. DNA sequence data were assembled and edited using the Lasergene sequence analysis software (DNASTAR Inc., Madison, WI). Sequence homology searches were performed using the basic local alignment search tool (BLAST) program (30) against the National Center for Biotechnology Information (NCBI) DNA database. The *porB* gene of samples that did not bind probes at the majority of VRs or showed mixed alleles were sequenced and submitted as AU26, AU15, AU27, AU54, and AU63 to the GenBank database under accession numbers DQ295202–DQ295206, respectively.

porB VR Typing Nomenclature

Hybridization results for a single VR are referred to as the "VR type," designated by the Por class, the VR, and the probe; e.g., PIB1-2. When more than one probe is bound for a single VR, each probe is listed separated by a slash (e.g., PIB5-3/5/6). The "*porB* type" includes results for all VRs tested, listed sequentially and separated by a semicolon; for example, B2;2;7;1/2/3;1 refers to a strain hybridizing PIB1-2, PIB3-2, PIB5-7, PIB6-1, PIB6-2, PIB6-3, and PIB7-1 probes. No hybridization signal detected for an individual VR is indicated by "nt".

RESULTS

PCR Amplification of *N. gonorrhoeae*

Nested PCR amplification resulted in a single band of approximately 975 bp for 39 of 43 (91%) of the stored frozen extracted DNA samples. The four samples that did not amplify *porB* were retested using the same *cpxB* assay as used originally (28). Two samples failed to amplify the *cpxB* gene and two samples were positive for the *cpxB* gene (data not shown).

porB VR Typing by PCR ELISA

The *porB* VR types of *N. gonorrhoeae* control strains MS11, 3744, 9299, and 2432 were characterized using 23

PIB VR probes and strains 252, 177007, PI83, DGI27, and 192 were characterized using 17 PIA VR probes (Table 1). For the PIB strains, the types obtained for VRs 3, 5, and 6 using ELISA were the same as that obtained using checkerboard hybridization. However, VRs 1 and 7 varied in the intensity of hybridization signal measured using ELISA and checkerboard hybridization and affected the genotype determined for the control strains (Table 1). For the PIA strains there was also variation in signal intensity observed at VRs 1, 2, 3, and 6 between PCR ELISA and checkerboard hybridization (Table 1). The *porB* VR types of clinical samples were determined using all 40 probes for all samples. Among the 39 PCR amplified clinical samples, 35 had one of eight PIB PCR-ELISA VR types. The most common type, B 2;2;7;4;2, was present in 12 samples from 10 MSM (34% prevalence), and type B 2;1;2;2;3, was present in seven samples from seven MSM (24% prevalence). The six other *porB* types were each identified in one to three samples (3–10% prevalence). No correlation was observed between the VR type of the PIB alleles and the anatomical sites where the samples were collected (Table 1). No samples were identified as PIA *N. gonorrhoeae. porB* from four samples showed only weak binding of a PIA probe for one or two VRs. These were confirmed by hybridization and *porB* sequencing to be *N. meningitidis*. No mixed *porB* types were observed in the 35 samples with PIB alleles, although two of 35 samples had mixed PIB *porB* and *N. meningitidis porB* amplified.

Two subjects provided two samples each: samples 6 and 7, and samples 9 and 10. Both samples from each patient gave the same VR type by PCR ELISA. The VR type B (2;2;7;1/2/3;1) was obtained from one individual that provided two rectal and two throat swabs (samples 16, 17, 18, and 19) and another individual who provided samples 14 (rectal) and 15 (throat). Thus, gonococcal *porB* DNA from 35 clinical samples from 29 MSM were used to compare VR typing by PCR ELISA and checkerboard hybridization.

porB VR Typing by Checkerboard Hybridization

Among the 39 clinical samples in which *porB* was amplified, no PIA, 35 PIB and 4 *N. meningitidis porB* samples were identified. Overall, there were 11 PIB *porB* VR types. Four *porB* types differed only by the loss of hybridization signal of the VR-5 probe, the VR-6 probe or both (Table 1). Taken together, this group of *porB* types accounted for 13 samples from 11 individuals (38%). The *porB* type B 2;1;2;2;3 was identified in seven samples from seven individuals (24%), and B 2;2;7;2;1 in six samples from two individuals.

TABLE 1. *porB* VR types of *N. gonorrhoeae* control strains and specimen samples*

Sample	Anatomical site	Por VR type	
		PCR ELISA	Checkerboard hybridization
1	Rectal swab	B 2;2;7;4;2	B 2;2;7;4;2
2	Rectal swab	B 2;2;7;4;2	B 2;2;7;4;2
3	Rectal swab	B 2;2;7;4;2	B 2;2;nt;4;2
4	Throat swab	B 2;2;7;4;2	B 2;2;nt;4;2
5	Throat swab	B 2;2;7;4;2	B 2;2;nt;4;2
6 ^a	Urine	B 2;2;7;4;2	B 2;2;nt;nt;2
7 ^a	Urine	B 2;2;7;4;2	B 2;2;nt;nt;2
8	Rectal swab	B 2;2;7;4;2	B 2;2;nt;nt;2
9	Rectal swab	B 2;2;7;4;2	B 2;2;nt;nt;2
10 ^b	Throat swab	B 2;2;7;4;2	B 2;2;nt;nt;2
11 ^b	Rectal swab	B 2;2;7;4;2	B 2;2;7;nt;2
12	Rectal swab	B 2;2;7;4;2	B 2;2;7;nt;2
13	Throat swab	B 2;2;7;nt;2	B 2;2;7;nt;2
14 ^c	Throat swab	B 2;2;7;(1/2/3);1	B 2;2;7;2;1
15 ^c	Rectal swab	B 2;2;7;(1/2/3);1	B 2;2;7;2;1
16 ^d	Rectal swab	B 2;2;7;(1/2/3);1	B 2;2;7;2;1
17 ^d	Rectal swab	B 2;2;7;(1/2/3);1	B 2;2;7;2;1
18 ^d	Throat swab	B 2;2;7;(1/2/3);1	B 2;2;7;2;1
19 ^d	Throat swab	B 2;2;7;(1/2/3);1	B 2;2;7;2;1
20	Throat swab	B 2;2;2;(1/2/3);1	B 2;2;nt;2;1
21	Rectal swab	B 2;2;2;(1/2/3);1	B 2;2;nt;2;1
22	Throat swab	B 2;2;2;(1/2/3);1	B 2;2;nt;2;1
23	Throat swab	B 2;1;2;2;3	B 2;1;2;2;3
24	Rectal swab	B 2;1;2;2;3	B 2;1;2;2;3
25	Throat swab	B 2;1;2;2;3	B 2;1;2;2;3
26	Rectal swab	B 2;1;2;2;3	B 2;1;2;2;3
27	Throat swab	B 2;1;2;2;3	B 2;1;2;2;3
28	Rectal swab	B 2;1;2;2;3	B 2;1;2;2;3
29	Throat swab	B 2;1;2;2;3	B 2;1;2;2;3
30	Rectal swab	B 2;2;(3/5/6);5;3	B 2;2;nt;5;3
31	Rectal swab	B 2;2;(3/5/6);5;3	B 2;2;nt;5;3
32	Rectal swab	B 2;2;(3/5/6);5;3	B 2;2;nt;5;3
33	Throat swab	B 3;1;4;4;2	B 3;1;4;4;2
34	Rectal swab	B 3;1;4;4;2	B 3;1;nt;4;2
35	Rectal swab	B 3;1;9;4;1	B 3;1;9;4;1
2432	PIB control strain	B (1/3);1;9;4;(1/3)	B 3;1;9;4;1
3744	PIB control strain	B 2;2;4;(4/6);nt	B 2;2;4;(4/6);2
9299	PIB control strain	B (1/3);1;1;3;(1/3)	B (3);1;1;3;(1/3)
MS11	PIB control strain	B 3;nt;8;5;3	B 1;1;8;5;3
252	PIA control strain	A 2;4;(1/3);3;3	A 2;4;3;3;3
177007	PIA control strain	A 1;(2/4);(1/2);1;1	A 1;2;1;1;1
PI83	PIA control strain	A 1;1;(1/2);2;1	A (1);1;1/2;2;3;1
DGI27	PIA control strain	A 1;3;1;1;1	A 1;3;1;1/4;1
192	PIA control strain	A (1/3);1;(1/2);2;2	A 3;1;2;2;2

*PIA types included VRs 1, 2, 3, 6, and 7. PIB types included VRs 1, 3, 5, 6, and 7. Samples have been renumbered and grouped according to genotype.

^{a-d}Indicate multiple samples collected from the same patient.

Comparison of PCR ELISA and Checkerboard Hybridization

The PCR ELISA results were compared to *porB* types obtained using checkerboard hybridization. There was exact agreement between the two methods for typing PIB

VRs 1, 3, and 7 for all 35 PIB samples. In VRs 5 and 6, no contradictory results were obtained, but some differences in the stringency of the hybridization reactions were observed between the two methods (Table 1). Some samples that were otherwise identical for VRs 1, 3, and 7 did not hybridize to the VR 5 or 6 probe in the checkerboard assay although PCR ELISA detected a signal. For some probes, a higher OD was observed in samples that hybridized by both methods than for samples that only hybridized the probe by PCR ELISA. For example, the PCR ELISA OD values for probe PIB6-4 ranged from 2.55 to 3.38 for samples that were B (2;2;7;4;2) by the checkerboard method, and ranged from 0.86 to 1.87 for samples that were B (2;2;7;nt;2) or B (2;2;nt;nt;2) by checkerboard hybridization. However, such trends were not observed for other probes.

PCR ELISA showed hybridization of some samples to several probes for the same VR that differed by only 1–2 bp. It was not possible to differentiate the exact VR type using probes PIB5-3, PIB5-5, and PIB5-6 and these were classified as 3/5/6 in Table 1. By checkerboard hybridization these samples showed no binding with any loop 5 probe. VR probes PIB6-1, PIB6-2, and PIB6-3 also displayed cohybridization and these were classified as 1/2/3 in samples 14 to 22 (Table 1). In the checkerboard assay, these samples were VR typed PIB6-2.

Two samples, samples 25 and 29, contained PIB gonococcal *porB* DNA and also hybridized probes PIA3-3 and/or PIA7-3. Amplicons from these samples, and the four samples that hybridized no PIB probes were tested using a similarly designed set of probes corresponding to the VR of the *porB* gene of *N. meningitidis*. Probes for each VR of class 3 meningococcal *porB* were hybridized by all six samples (data not shown). These results suggest the samples 25 and 29 contained both gonococcal and meningococcal *porB* DNA. Both samples were collected from the throat.

Sequence Characterization of the *porB* Gene

Samples 30, 31, and 32 that demonstrated cohybridization of several VR5 probes were selected for sequence

analysis. The VR5 nucleotide sequence differed from probes PIB5-3, PIB5-5, and PIB5-6 by either one of two adjacent nucleotides localized to the center of the probe hybridization site (Table 2). The sequence data show that probe hybridization occurs in the ELISA method but not in the checkerboard method in the presence of single base differences between the sample DNA and the VR5-3, 5, and 6 probes.

Sequence analysis of the *porB* gene from samples 25 and 29, which contained both *N. gonorrhoeae* and *N. meningitidis porB* DNA was performed. Sample 25 displayed 99% nucleotide identity over 271 bp with a *N. gonorrhoeae porB* gene (Ac: AY765446). Sample 29 displayed 98% sequence identity with a *N. meningitidis porB* gene over 682 bp using sequencing primers PIB.F, PIB.R, and GCseq.F471. Sequence analysis of the four samples with amplified *porB* that did not bind PIB or PIA probes for the majority of VRs resulted in sequences (GenBank DQ295202-DQ295206) with homology to published *porB* class 3 genes from *N. meningitidis*.

DISCUSSION

Both checkerboard and PCR ELISA hybridization assays were able to characterize the gonococcal infections of 29 MSM using direct clinical samples from urine, rectal, and throat swabs. By PCR-ELISA, approximately 58% of the PIB strains had one of two prevalent *porB* types and six other types were represented in the remaining samples, indicating limited number of circulating types in this population. Overall, checkerboard hybridization was more discriminatory than PCR ELISA. However, binding of probes in the PCR ELISA indicated that the samples that were similar to the predominant B (2;2;7;4;2) type, but were not typed in VR 5 and 6 by checkerboard hybridization, likely had only minor sequence variations in these regions. PCR ELISA provided additional data regarding those VRs that were not typed by checkerboard hybridization, and provided evidence that the strains that were suspected as having similar sequences based

TABLE 2. The sequence of the loop 5 VR of the *porB* gene amplified from samples 30, 31, and 32*

Accession number or sample number	<i>Por</i> class and probe	Sequence (5' to 3')
AF044794	PIB5-3	AAAAAAATGGAAGGATATCTATATAATATCCCCAGT
AY765442	PIB5-5	AAAAAAATGGAAGGATATGCATATAATATCCCCAGT
NA	PIB5-6	ATGGAAGGATATGCATATAATATCCCCAG
Sample 30		AAAAAAATGGAAGGATATCCATATAATATCCCCAGT
Sample 31		AAAAAAATGGAAGGATATTCATATAATATCCCCAGT
Sample 32		AAAAAAATGGAAGGATATTCATATAATATCCCCAGT

*Sequences were aligned against the sequence of probes PIB5-3, PIB5-5, and PIB5-6 for comparison. Polymorphic nucleotides are highlighted. NA, no accession number available.

on the other VRs, had very similar sequences in the nontyped VRs. These results suggest that the two methods as performed here result in different levels of stringency for probe binding. As a result, PCR-ELISA was more sensitive for probe binding in VRs 5 and 6, while checkerboard hybridization was more specific for identifying sequence identity to VR probes.

One of the potential advantages of the PCR ELISA method is that the hybridization signal is quantifiably measured and hence specificity may be improved by having stepwise cutoffs for high binding (implying complete sequence identity) and moderate binding (with close sequence identity but with likely base pair changes). The signal intensity for samples from direct clinical specimens may also be affected by variations in the bacterial load or DNA from other *Neisseria* species. As such, further VR typing studies using ELISA may look at using *N. gonorrhoeae* specimens derived from and in parallel with direct clinical samples to measure the potential variation in signal intensity associated with template copy number or bacterial load. This work may allow better optimization of this method and allow for increased specificity while maintaining the sensitivity observed using PCR-ELISA.

In this study, four samples were excluded from this comparison because they failed to amplify *porB*. This is a low rate given that the samples were direct clinical specimens that had been previously obtained and stored. Nested *porB* amplification may have failed due to DNA degradation since the last sample collection in September 2002 (2). The *cpxB* assay being more sensitive in detecting *N. gonorrhoeae* than *porB* amplification could contribute to lack of amplification from the same samples, and it is also possible that the *cpxB* assay amplified a product from another *Neisseria* species (2).

Four additional samples did not hybridize with *porB* VR probes for most VRs even though PCR products were obtained. These samples were shown by hybridization of specific probes and by sequencing to contain *N. meningitidis porB*. Identification of *N. gonorrhoeae* using PCR from noncultured samples from the throat and rectum has been complicated by a lack of specificity and potential cross reactivity with other *neisseria* species prevalent at these sites (31). Recently, two methods utilizing amplification of the *opa* and *porA* pseudogene have been shown to be sensitive and specific (29,32). However, few molecular methods for typing strains from direct clinical specimens have been reported and NAAT methods do not allow for further identification of strain type.

Two important goals for typing *N. gonorrhoeae* from direct clinical samples are the identification of coinfection with more than one strain and the ability to distinguish *N. gonorrhoeae* from other closely related

Neisseria species. *N. meningitidis* is especially common in samples collected from the throat (33). In this study, meningococcal *porB* was amplified alone in four samples and in combination with gonococcal *porB* in two samples. In both circumstances, accurate identification was possible by probe hybridization. In the two samples that showed hybridization patterns indicating the presence of both *N. gonorrhoeae* and *N. meningitidis*, the *porB* sequence obtained for one was homologous to gonococcal *porB* and for the other sample, the sequence obtained was similar to *N. meningitidis porB*. Accurate sequencing from samples that have both gonococcal and meningococcal *porB* DNA will require selection of sequencing primers that differentiate each species but are conserved within each species. The samples that contained *N. meningitidis porB* DNA displayed a pattern of binding probes PIA 7-3 and or PIA 3-3. Binding of the PIA3-3 probe may be due to high similarity in the loop 3 region of the *N. meningitidis porB* gene of strains typically typed as serotype 4, and the lack of probe hybridization for the majority of VRs distinguished these from typical PIA *N. gonorrhoeae*. PCR-ELISA has the ability both to detect the presence of other *Neisseria* species in direct clinical samples and successfully characterize *N. gonorrhoeae* in the presence of these species.

The current probe panel identified *porB* types for all samples with amplified gonococcal *porB* DNA. However, future sequence analysis of PCR amplified *porB* gene can be used to develop additional probes suitable for typing as needed. The cohybridization of PIB probes PIB5-3, PIB5-5, and PIB5-6 such as seen in samples 30, 31, and 32 could be further modified by utilizing sequenced control strains and by designing new probes that specifically target the polymorphic nucleotides identified in this region.

The results of this evaluation indicate that PCR ELISA can rapidly distinguish VRs in the *N. gonorrhoeae porB* gene in clinical samples. In addition, by utilizing hybridization, this method can type samples that contain other *Neisseria* species found in extra-genital samples such as throat samples. PCR ELISA has broad applicability, as there is no need for expensive equipment or reagents, hence making it suitable for most laboratories and clinics. PCR ELISA demonstrates potential for being a valuable procedure for typing *N. gonorrhoeae* strains including noncultured specimens used for NAAT diagnostic methods.

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