JOURNAL OF CLINICAL MICROBIOLOGY, May 2006, p. 1887–1891 Vol. 44, No. 5
0095-1137/06/$08.00+0 doi:10.1128/JCM.44.5.1887–1891.2006


# Multiplex PCR-Based Reverse Line Blot Hybridization Assay To Identify 23 *Streptococcus pneumoniae* Polysaccharide Vaccine Serotypes

Fanrong Kong,[1] Mitchell Brown,[1,2] Archcna Sabananthan,[1,3] Xianyu Zeng,[1,4] and Gwendolyn L. Gilbert[1]*

*Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research, Westmead, New South Wales, Australia[1]; Department of Cell and Molecular Biology, Faculty of Science, University of Technology, Sydney, New South Wales, Australia[2]; School of Biotechnology and Biomolecular Sciences, The University of New South Wales, New South Wales, Australia[3]; and Wuhan First Hospital, Hubei Province, Wuhan 430022, People's Republic of China[4]*

Received 11 November 2005/Returned for modification 28 January 2006/Accepted 27 February 2006

**We developed a multiplex PCR-based reverse line blot assay to identify 23 pneumococcal serotypes represented in the polysaccharide vaccine, using 334 well-characterized isolates, representing all 90 serotypes, and 268 "unknowns." The assay identified all target serotypes, but 11, which cross-react with 1 to 4 nonvaccine serotypes, could be distinguished using serotype-specific antisera.**

Previously, we developed a molecular capsule type prediction system for 90 *Streptococcus pneumoniae* serotypes, based on a combination of partial *cpsA-cpsB* sequencing and serotype- and/or serogroup-specific PCR (3, 6). While this system is useful, it is too slow, expensive, and labor-intensive for routine use.

Multiplex PCR-based reverse line blot hybridization (mPCR/RLB) is a promising method for simultaneous detection and genotyping of microorganisms, which we have used previously in several applications (5, 9, 10). In the present study we applied it to identification of the 23 *S. pneumoniae* serotypes represented in the polysaccharide vaccine (Pneumovax 23; Merck & Co, Inc.).

The design of primers and probes was based on recently published full *cps* gene cluster sequences of all 90 pneumococcal serotypes (http://www.sanger.ac.uk/Projects/S_pneumoniae/CPS/) and others available in GenBank (3, 6; F. Kong, G. L. Gilbert, L. Wang, D. Liu, and J. Tao, 13 April 2004, Australian Patent Office). For the mPCR, we modified the primers we had used previously for serotype-specific PCR for the 23 vaccine serotypes (3, 6; Kong et al., Australian Patent Office) and a published *S. pneumoniae*-specific primer (7). For the RLB, we designed two probes for each of the 23 serotypes, as well as two *S. pneumoniae*-specific control probes (Table 1). Probes and primers were designed to have similar physical characteristics so as to allow simultaneous amplification and hybridization in a multiplex reaction system (5); they were synthesized by Sigma-Aldrich (Sydney, Australia). Primers were biotinylated at the 5′ end and probes had a 5′-amine group (5).

The DNA extraction method (4), the mPCR system, and the thermal profiles used were as previously described (5), except that we included 24 (rather than 10) primer pairs (Table 1) in a 25-μl mPCR system and used 1 U (rather than 0.5) of QIAGEN Hotstart *Taq* polymerase. For serotype 23F, 25 pM of each primer were used, whereas 12.5 pM of each primer was used for all other serotypes.

RLB hybridization was based on previously published methods (http://www.nioo.knaw.nl/cl/me/) (8, 11) with the following modifications: the hybridization temperature was 60°C, and 1.25 pM of each probe, in 150 μl of 500 mM $NaHCO_3$ (pH 8.4), was used in each slot to label the membrane (Fig. 1).

A collection of 334 reference strains and well-characterized clinical isolates were used to develop the assay, of which 244 had been used in our previous studies (3, 6) and 90 were serotype reference strains, newly purchased from Statens Serum Institut of Denmark (1, 2). Except for serotypes 10C, 11F, 12B, 25A, 33D, and 44, all serotypes were represented by two or more strains.

All of the putative serotype-specific primers or probes yielded mPCR products and RLB signals from isolates with the corresponding serotypes. However, as demonstrated previously, serotype discrimination based solely on the *wzy* gene (or even the whole *cps* gene cluster) is not straightforward (3, 6). Primers and probes designed to identify serotypes 6B, 7F, 9N, 9V, 10A, 11A, 12F, 15B, 18C, 22F, and 33F could not distinguish between the target and one or more closely related serotypes, usually but not always in the corresponding serogroup (Table 1 and Fig. 1) because they share virtually identical *wzy* sequences. Thus, 17 serotypes, in addition to those in the 23-valent polysaccharide vaccine, were amplified and hybridized by the mPCR/RLB system. These cross-reactions are predictable, and individual serotypes can be identified using a limited number of factor antisera. The remaining 12 primer pairs and probe pairs were truly serotype specific, and there was excellent agreement between paired probes for the same serotypes, indicating that single probes would be adequate for most serotypes.

The method was further evaluated using 268 clinical isolates, the serotypes of which were unknown at the time of mPCR/RLB testing. These isolates included 135 consecutive invasive isolates referred to the NSW Pneumococcal Reference Laboratory for serotyping and 133 colonizing isolates from patients with respiratory infections at the Children's Hospital, West-

* Corresponding author. Mailing address: Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital, Darcy Road, Westmead, New South Wales 2145, Australia. Phone: (612) 9845-6255. Fax: (612) 9893-8659. E-mail: lyng@icpmr.wsahs.nsw.gov.au.

TABLE 1. Oligonucleotide primers and probes for multiplex PCR-based reverse line blot assay used in this study

| Primer[a] | Target | Specificity[b] | $T_m$ (°C)[c] | GenBank accession no. | Sequence (5′ to 3′)[d] |
|---|---|---|---|---|---|
| SpIaSb[e] | Pneumolysin | *S. pneumoniae* | 61.43 | M17717 | **384**ATT TCT GTA ACA GCT ACC AAC GA**406** |
| SpSp | Pneumolysin | *S. pneumoniae* | 65.49 | M17717 | **531**AGC GAT AGC TTT CTC CAA GTG G**552** |
| SpAp | Pneumolysin | *S. pneumoniae* | 75.10 | M17717 | **556**ACC CCA GCA ATT CAA GTG TTC GCG**579** |
| SpIbAb[e] | Pneumolysin | *S. pneumoniae* | 61.69 | M17717 | **730**GAA TTC CCT GTC TTT TCA AAG TC**708** |
| 1Sb | *wzy* | Serotype 1 | 59.03 | Z83335 | **10307**GGG ACT TTA ATT TTA TGC AGT G**10328** |
| 1Ap | *wzy* | Serotype 1 | 59.22 | Z83335 | **10402**AAA TTT CAC AAT TAT CAT TGC C**10381** |
| 1Sp | *wzy* | Serotype 1 | 62.00 | Z83335 | **10529**CTG GCT TTG GCA ACT TTG**10546** |
| 1Ab | *wzy* | Serotype 1 | 60.67 | Z83335 | **10576**CAC AAT GGC TTT AGA AGG TAG AG**10554** |
| 2Sb | *wzy* | Serotype 2 | 59.86 | AF026471 | **9730**CGG CAT TGT ATT CTT TAT ATC G**9751** |
| 2Ap | *wzy* | Serotype 2 | 60.40 | AF026471 | **9849**CCA ATA AAT CTT GTG TGA ATA TAA CTG**9823** |
| 2Sp | *wzy* | Serotype 2 | 60.76 | AF026471 | **9989**GCA ACA TTT CAA TCT TAT GGT G**10010** |
| 2Ab | *wzy* | Serotype 2 | 63.05 | AF026471 | **10049**CGT TTG TAT CCA TTT AAC TGC ATC**10026** |
| 3Sb | *wze* | Serotype 3 | 59.32 | Z47210 | **5807**TTG ATA TTC CCC TTG ACA ATA G**5828** |
| 3Sp | *wze* | Serotype 3 | 59.62 | Z47210 | **5910**TTT ACT ACA GTC CTC TTT CTC TGC**5887** |
| 3Ap | *wze* | Serotype 3 | 58.63 | Z47210 | **6056**GCC TCA TCC TTA ATT GGA G**6038** |
| 3Ab | *wze* | Serotype 3 | 61.69 | Z47210 | **6102**GGA GGC TTC AAG ATT CAA CTC**6082** |
| 4Sb | *wzy* | Serotype 4 | 61.09 | AF316639 | **9619**CCG TCT ATA TTT ATA TGG GTT TGC**9642** |
| 4Ap | *wzy* | Serotype 4 | 60.08 | AF316639 | **9774**TTG AAA CCC CAT ATA CTC ATT G**9753** |
| 4Sp | *wzy* | Serotype 4 | 60.49 | AF316639 | **9848**GGA TTT TGT TTT GTT ATT CTG TAG G**9872** |
| 4Ab | *wzy* | Serotype 4 | 59.48 | AF316639 | **9934**CCT GAT AAT TTT GTA CTT CTG AAT G**9910** |
| 5Sb | *wzy* | Serotype 5 | 61.23 | AY336008 | **6052**GTT TTC CCA ATA GTA GTT TGC G**6073** |
| 5Ap | *wzy* | Serotype 5 | 61.86 | AY336008 | **6118**TGC GAT AAA ATA GAT AAG GCA ATC**6095** |
| 5Sp | *wzy* | Serotype 5 | 62.20 | AY336008 | **6275**TGC TTT GTT AGA TAT AGT TAC GGG AG**6300** |
| 5Ab | *wzy* | Serotype 5 | 62.04 | AY336008 | **6349**CCC ACA GCC AAA TAG AGT TG**6330** |
| 6B6ASb | *wzy* | Serogroup 6 (**6A**, 6B) | 58.77 | AF316640 | **9263**TCA ACC TGC AGT AAT TTT AAC A**9284** |
| 6B6AAp | *wzy* | Serogroup 6 (**6A**, 6B) | 60.95 | AF316640 | **9322**TTA ACT AGA GCA CTT GCA ATC G**9301** |
| 6B6Asp | *wzy* | Serogroup 6 (**6A**, 6B) | 59.01 | AF316640 | **9461**GAA AGA AAT AAA TCC TTC AAA GAT AAT**9487** |
| 6B6AAb | *wzy* | Serogroup 6 (**6A**, 6B) | 60.19 | AF316640 | **9555**CTA CTT TCT GAA TTT CAC GGA TAT AAA G**9528** |
| 7F7ASb | *wzy* | Serotypes 7F, **7A** | 61.48 | CR931643 | **14476**GCA AGT GTT TCA ATG GGA GTA**14496** |
| 7F7AAp | *wzy* | Serotypes 7F, **7A** | 58.71 | CR931643 | **14537**AAA TTC CAC AAT ATT GGT AAT ACT G**14513** |
| 7F7Asp | *wzy* | Serotypes 7F, **7A** | 58.58 | CR931643 | **14776**TTT TTG TAT GAT TTA AAT ATT GCG**14799** |
| 7F7AAb | *wzy* | Serotypes 7F, **7A** | 61.51 | CR931643 | **14820**ACG GAG GGA CCA TAC AAT AAG**14800** |
| 8Sb | *wzy* | Serotype 8 | 60.50 | AF316641 | **10827**GAT GTT AGT TTC TTC GAT TCC AG**10849** |
| 8Ap | *wzy* | Serotype 8 | 59.15 | AF316641 | **10870**GAG GAA ACC CAC AAA GTC AAA AAA C**10849** |
| 8Sp | *wzy* | Serotype 8 | 59.38 | AF316641 | **11012**AAA ATT ATG TTT ACT TTA CGA GTT GG**11037** |
| 8Ab | *wzy* | Serotype 8 | 61.32 | AF316641 | **11066**TCA TAA TGA ATC GTA CCA ATC AAC**11043** |
| 9N9LSb | *wzy* | Serotypes 9N, **9L** | 62.46 | CR931647 | **9818**TCA ATG GCG ACT TTA TTT GC**9837** |
| 9N9LAp | *wzy* | Serotypes 9N, **9L** | 58.53 | CR931647 | **9893**GAA CTT TGG GAA TAT AAT CAA AAG**9870** |
| 9N9LSp | *wzy* | Serotypes 9N, **9L** | 62.15 | CR931647 | **10139**GTC GGT TTC GAT TCT TTG C**10157** |
| 9N9LAb | *wzy* | Serotypes 9N, **9L** | 60.41 | CR931647 | **10179**AGT CTA TTA TCT CCT GTA GGG TGC**10156** |
| 9V9ASb | *wzy* | Serotypes 9V, **9A** | 58.70 | AF402095 | **8546**AAC TAT ATT TAC CCT ACT CTC CAC AG**8571** |
| 9V9AAp | *wzy* | Serotypes 9V, **9A** | 58.76 | AF402095 | **8639**AAT CAG CGT TAC CTT ATA CTG TG**8617** |
| 9V9ASp | *wzy* | Serotypes 9V, **9A** | 59.62 | AF402095 | **8797**GCC TCT TTT TAA CCT TTA TCT TGT**8820** |
| 9V9AAb | *wzy* | Serotypes 9V, **9A** | 61.56 | AF402095 | **8860**ACC GGA AAA AGC AAT TGA G**8842** |
| 10A10BSb | *wzy* | Serotypes 10A, **10B** | 61.86 | CR931649 | **7172**TGA GCT ATT TAA GGA CCT GGG**7192** |
| 10A10BAp | *wzy* | Serotypes 10A, **10B** | 62.30 | CR931649 | **7206**GTT TAG AAA CCT TGC CCA GG**7187** |
| 10A10BSp | *wzy* | Serotypes 10A, **10B** | 62.25 | CR931649 | **7353**ACC ATA TGG TAT TTG TTG CCT G**7374** |
| 10A10BAb | *wzy* | Serotypes 10A, **10B** | 61.76 | CR931649 | **7450**GCA AGC GTC ACT TTC TTG A**7432** |
| 11A11DSb | *wzy* | Serotypes 11A **11D** | 59.48 | CR931653 | **11368**GAA ATA TCG CCA TTC ATC AG**11387** |
| 11A11DAp | *wzy* | Serotypes 11A, **11D** | 59.34 | CR931653 | **11407**TGT AAG ATG AAA AAA GCA TGC**11387** |
| 11A11DSp | *wzy* | Serotypes 11A, **11D** | 59.42 | CR931653 | **11676**GGA TTT CTC GTA TCA GCA TAT TAC**11699** |
| 11A11DAb | *wzy* | Serotypes 11A, **11D** | 62.68 | CR931653 | **11744**CAA CAG CAA CTG TGC CAC T**11726** |
| 124446Sb | *wzy* | Serotypes 12F, **12A, 12B**, **44, 46** | 59.41 | CR931660 | **8914**TGA ATA TGG ACG GTG GAG**8931** |
| 124446Ap | *wzy* | Serotypes 12F, **12A, 12B**, **44, 46** | 58.48 | CR931660 | **8951**GAA GAA GTT CAA CAA TCG CT**8932** |
| 124446Sp | *wzy* | Serotypes 12F, **12A, 12B**, **44, 46** | 59.37 | CR931660 | **9104**GCA TGA TAT GGG AAG TTT TG**9123** |
| 124446Ab | *wzy* | Serotypes 12F, **12A, 12B**, **44, 46** | 59.69 | CR931660 | **9155**AGC AAA GAA AGC CGA AAG**9138** |
| 14Sb | *wzy* | Serotype 14 | 60.41 | X85787 | **7376**CCT ACT TCC AAA ACA GTT TAT GC**7398** |
| 14Ap | *wzy* | Serotype 14 | 59.77 | X85787 | **7494**CCA TAC AAA AAG ACG GTG TAT C**7473** |
| 14Sp | *wzy* | Serotype 14 | 59.30 | X85787 | **7587**GCA TTT AAT TGG CTA ATA GCA G**7608** |
| 14Ab | *wzy* | Serotype 14 | 59.69 | X85787 | **7652**GTC AAT ATT GAT TGG CAT TTT C**7631** |
| 15B15CSb | *wzy* | Serotypes 15B, **15C** | 62.63 | CR931664 | **7797**TAA TAA GCG GAT GAT TGT AGC G**7818** |
| 15B15CAp | *wzy* | Serotypes 15B, **15C** | 58.19 | CR931664 | **7837**GAG GTA TAG TTG GAT AAA ACG C**7816** |

*Continued on following page*

TABLE 1—*Continued*

| Primer[a] | Target | Specificity[b] | $T_m$ (°C)[c] | GenBank accession no. | Sequence (5′ to 3′)[d] |
|---|---|---|---|---|---|
| 15B15CSp | *wzy* | Serotypes 15B, **15C** | 62.54 | CR931664 | **7976**GAG CAG GAA TCA GAA CAC AAT C**7997** |
| 15B15CAb | *wzy* | Serotypes 15B, **15C** | 60.91 | CR931664 | **8148**TAT ACT GAT TAA CTT TCC AGA TGG G**8124** |
| 17FSb | *wzy* | Serotype 17F | 59.84 | CR931670 | **13988**AGA GGG ATT GTT GAA GGT ATT C**14009** |
| 17FAp | *wzy* | Serotype 17F | 60.89 | CR931670 | **14035**GGA AGT GAA CGT CAA ATC TTT T**14014** |
| 17FSp | *wzy* | Serotype 17F | 61.75 | CR931670 | **14250**CAA TGC TAT GTC GCA AAT ATT G**14271** |
| 17FAb | *wzy* | Serotype 17F | 61.34 | CR931670 | **14295**AGT AGT CTC GCA TTT CTA TCA TCC**14272** |
| 18Sb | *wzy* | Serogroup 18 (**18F, 18A, 18B,** 18C) | 60.45 | AF316642 | **12208**AAT TGT TCT TTT CCT GTA CTC AGT C**12232** |
| 18Ap | *wzy* | Serogroup 18 (**18F, 18A, 18B,** 18C) | 58.72 | AF316642 | **12260**ATT CAA C/TTG GGT TCA TTA CG**12241** |
| 18Sp | *wzy* | Serogroup 18 (**18F, 18A, 18B,** 18C) | 58.74 | AF316642 | **12425**GGA GGA CTT AGT CAA TTT ATC TTG**12448** |
| 18Ab | *wzy* | Serogroup 18 (**18F, 18A, 18B,** 18C) | 62.13 | AF316642 | **12478**CGA ACC ATT GAA ACT ATC ATC TG**12456** |
| 19ASb | *wzy* | Serotype 19A | 61.40 | AF094575 | **9260**TGT ATT TGC CCT TAT TAA TGT GC**9282** |
| 19AAp | *wzy* | Serotype 19A | 61.12 | AF094575 | **9336**TGC CAC TAA TAA TCA AAA GAT AAG C**9312** |
| 19ASp | *wzy* | Serotype 19A | 60.81 | AF094575 | **9422**CCA ATT CTG GAA AAT AGC TCT TAC**9445** |
| 19AAb | *wzy* | Serotype 19A | 60.63 | AF094575 | **9506**AAG TGC AAG ATT ATG AAT CTC TCT C**9482** |
| 19FSb | *wzy* | Serotype 19F | 59.55 | U09239 | **7693**TCA GTA TTT GCA CTG GTT AAT TC**7715** |
| 19FAp | *wzy* | Serotype 19F | 61.85 | U09239 | **7800**TGC CAT TAA AGG AAT CGA AA**7781** |
| 19FSp | *wzy* | Serotype 19F | 60.35 | U09239 | **7858**CAA TTT TGG AAA ATT GCT CTA AC**7880** |
| 19FAb | *wzy* | Serotype 19F | 59.39 | U09239 | **7941**AAG AAC AAG GTT GTA TAT TTC CTT C**7917** |
| 20Sb | *wzy* | Serotype 20 | 61.18 | CR931679 | **7711**CTT TAT CAG GAA TAC GCC AAT C**7732** |
| 20Ap | *wzy* | Serotype 20 | 60.16 | CR931679 | **7759**GCA TAA AAA ACA ATC GCT GTA G**7738** |
| 20Sp | *wzy* | Serotype 20 | 62.31 | CR931679 | **7933**CTG GGT CTG AAT TTG TAT CTC G**7954** |
| 20Ab | *wzy* | Serotype 20 | 59.11 | CR931679 | **8010**CTG TAT AAT AAC GAG AAC CAA CG**7988** |
| 22F22ASb | *wzy* | Serotypes 22F, **22A** | 61.86 | CR931682 | **13190**AGG ATG CAG TAG ATA CCA GTG G**13211** |
| 22F22AAp | *wzy* | Serotypes 22F, **22A** | 59.68 | CR931682 | **13263**TAA TAC CAT GGC ACT AGG AAT AAC**13240** |
| 22F22ASp | *wzy* | Serotypes 22F, **22A** | 58.30 | CR931682 | **13503**ATG GCT ATC AAC TTT ATC TAG GAC**13526** |
| 22F22AAb | *wzy* | Serotypes 22F, **22A** | 60.25 | CR931682 | **13543**TAT AAA CGG AGG TTG TTG TCC**13523** |
| 23FSb | *wzy* | Serotype 23F | 60.39 | AF057294 | **8583**TGA TAG TGA ACT TGG GAT TGT C**8604** |
| 23FAp | *wzy* | Serotype 23F | 61.24 | AF057294 | **8694**CTA TTT GCA AAC ACG TTG AGA G**8673** |
| 23FSp | *wzy* | Serotype 23F | 58.36 | AF057294 | **8735**GGG ATT AAT TTA CAA AAT CTT CC**8757** |
| 23FAb | *wzy* | Serotype 23F | 58.19 | AF057294 | **8827**CTT TAT CGG TAA GGT GGA TAA G**8806** |
| 33F33A37Sb | *wzy* | Serotypes 33F, **33A, 37** | 61.77 | AJ006986 | **11362**TCA ACT AGT CAA GGA TTT GAT GG**11384** |
| 33F33A37Ap | *wzy* | Serotypes 33F, **33A, 37** | 60.62 | AJ006986 | **11421**TGA TAC CAC AAG TAA CAG AGT CG**11399** |
| 33F33A37Sp | *wzy* | Serotypes 33F, **33A, 37** | 59.52 | AJ006986 | **11589**GAT GAT TTT GCA GAT GTA CTA TGA**11612** |
| 33F33A37Ab | *wzy* | Serotypes 33F, **33A**, **37** | 59.67 | AJ006986 | **11639**CGT ATC AGA TTT GCG ATT TC**11620** |

[a] S, sense; A, antisense; b, biotin-labeled primer (primers were biotin labeled at the 5′ end); p, probe (probes were 5′ end C6 amine labeled).
[b] Based on published sequence data for the whole *cps* gene cluster, it was not possible to design primers/probes that could distinguish some individual serotypes from one or four closely related ones (shown in boldface), usually (but not always) belonging to the same serogroup.
[c] $T_m$ values were provided by the primer synthesizer (Sigma-Aldrich).
[d] Numbers represent the base positions at which primer/probe sequences start and finish (starting at "1" of the corresponding GenBank sequence).
[e] Two primers published previously (8) were used as species-specific probes.

mead, New South Wales, Australia. Conventional serotyping was performed using the Quellung reaction, as previously described (3). Two isolates were not amplified by mPCR/RLB, and phenotypic retesting showed that they were not *S. pneumoniae*. Of 266 pneumococcus strains, 12 (4.5%) were nontypeable by mPCR/RLB (amplified by mPCR but hybridized only with the pneumococcal control probe); of these, 10 belonged to serotypes not represented in the current mPCR/RLB assay, namely, 16F (one isolate), 23A (three isolates), 35F (four isolates), 35B (one isolate), and 38 (one isolate), and 2 were not serotypeable. Another 4 of the 266 pneumococcus isolates were not serotypeable but were identified by mPCR/RLB as serotype(s) 4, 11A/11D, 14, and 33F/33A/37. The predicted serotype(s) of the other 250 isolates were as follows: 3 (5 isolates), 4 (13 isolates), 6B/6A (45 isolates), 7F/7A (1 isolate), 9V/9A (15 isolates), 10A/10B (2 isolates), 11A/11D (2 isolates), 14 (51 isolates), 15B/15C (4 isolates), 18 (14 isolates), 19F (63 isolates), 19A (12 isolates), 22F/22A (2 isolates), and 23F (21 isolates). Thus, 181 of 254 (71%) isolates that were typeable by mPCR/RLB were identified exactly, and another 73 (29%) were identified to within one to five serotypes, for which a limited number of antisera were needed to distinguish individual serotypes.

Three isolates identified by mPCR/RLB as serotypes 19A, 9V/9A, and 23F had been serotyped, initially, as 19F, 19A, and 6B, respectively. The discrepancies were resolved by repeating the serotyping, which confirmed that the mPCR/RLB results were correct.

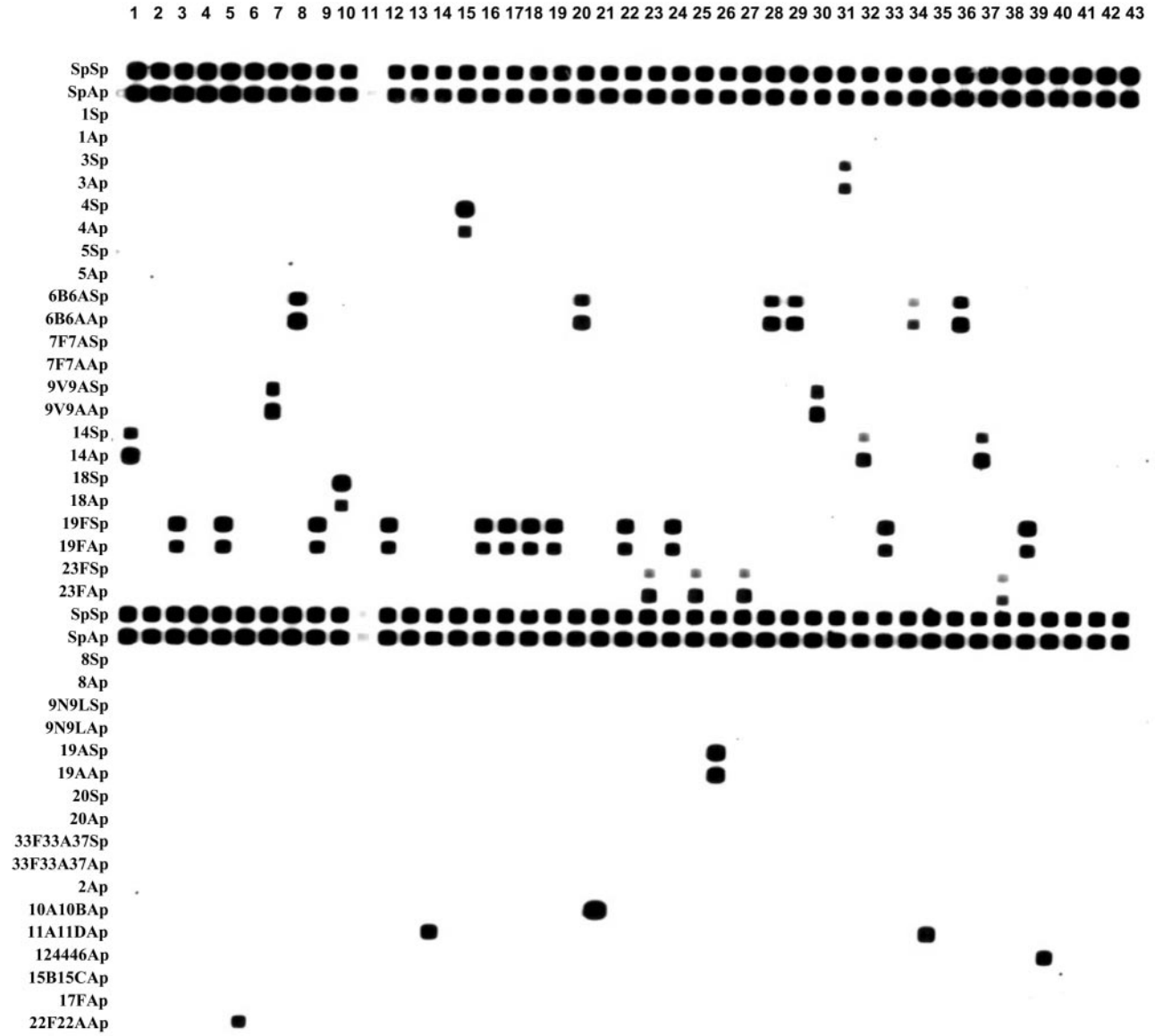


FIG. 1. mPCR/RLB results for a representative sample of 43 clinical isolates. See Table 1 for descriptions and specificities of probes listed at left. The choice of probes for this membrane, in addition to two pairs of *S. pneumoniae*-specific probes, was based on two for each of the serotypes represented in the 11-valent conjugate vaccine (serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F [shown in the top half of the membrane]) and others that are identified relatively commonly (serotypes 8, 9N, 19A, 20, and 33F) and single probes for each of the less common serotypes among those represented in the 23-valent vaccine (serotypes 2, 10A, 11A, 12, 15B, 17F, and 22F). The conventional serotypes of the 43 isolates shown on this membrane are (from left to right): 14, NT, 19F, NT, 19F, 22F, 9V, 6A, 19F, 18C, NSP, 19F, 16F, 11A, 4, 19F, 19F, 19F, 19F, 6B, 10A, 19F, 23F, 19F, 23F, 19A, 23F, 6A, 6B, 9V, 3, 14, 19F, 6B, 11A, 6A, 14, 23F, 19F, 46, 47F, 47A, and 48. NT, nontypeable. NSP, not *S. pneumoniae*.

Our molecular serotype prediction mPCR/RLB assay for 23 pneumococcal "vaccine" serotypes is clearly less discriminatory than conventional serotyping (1–3, 6; Kong et al., Australian Patent Office) because individual serotype-specific targets are not available within *cps* gene clusters of some serotypes. However, there are obvious advantages of the mPCR/RLB. First, it uses reagents and techniques that are available in many microbiology laboratories; an uncomplicated, rapid DNA preparation method (4); and a single mPCR/RLB reaction (5). Second, it provides more consistent and objective results than immunological methods such as the Quellung reaction; cross-reactions are predictable and can be resolved with a small number of antisera. Serotypes that are nontypeable by the mPCR/RLB are isolated uncommonly from clinical specimens (~5% of invasive isolates in NSW in the past 3 years [data not shown]). Third, the method, potentially, could to be used directly to test clinical specimens and so rapidly identify possible vaccine failure.

Currently, there is no single, ideal technique for pneumococcal serotyping, but this mPCR/RLB format is convenient, rapid, objective, reproducible, and discriminatory when used in conjunction with a limited number of antisera.

We thank Denise Murphy, Queensland Health Scientific Services, Brisbane, Australia; Diana Martin, Institute of Environmental Science Research, Porirua, New Zealand for providing isolates; and Ping Zhu for technical assistance.

This study was funded, in part, by the National Centre for Immunization Research and Surveillance of Vaccine Preventable Disease, Children's Hospital at Westmead, New South Wales, Australia.

## REFERENCES

1. **Henrichsen, J.** 1995. Six newly recognized types of *Streptococcus pneumoniae*. J. Clin. Microbiol. **33:**2759–2762.
2. **Henrichsen, J.** 1999. Typing of *Streptococcus pneumoniae*: past, present, and future. Am. J. Med. **107:**50S–54S.
3. **Kong, F., and G. L. Gilbert.** 2003. Using *cpsA-cpsB* sequence polymorphisms and serotype-/group-specific PCR to predict 51 *Streptococcus pneumoniae* capsular serotypes. J. Med. Microbiol. **52:**1047–1058.
4. **Kong, F., S. Gowan, D. Martin, G. James, and G. L. Gilbert.** 2002. Serotype identification of group B streptococci by PCR and sequencing. J. Clin. Microbiol. **40:**216–226.
5. **Kong, F., L. Ma, and G. L. Gilbert.** 2005. Simultaneous detection and serotype identification of *Streptococcus agalactiae* using multiplex PCR and reverse line blot hybridization. J. Med. Microbiol. **54:**1133–1138.
6. **Kong, F., W. Wang, J. Tao, L. Wang, Q. Wang, A. Sabananthan, and G. L. Gilbert.** 2005. A molecular-capsular-type prediction system for 90 *Streptococcus pneumoniae* serotypes using partial *cpsA-cpsB* sequencing and *wzy*- or *wzx*-specific PCR. J. Med. Microbiol. **54:**351–356.
7. **Salo, P., A. Ortqvist, and M. Leinonen.** 1995. Diagnosis of bacteremic pneumococcal pneumonia by amplification of pneumolysin gene fragment in serum. J. Infect. Dis. **171:**479–482.
8. **van den Brule, A. J., R. Pol, N. Fransen-Daalmeijer, L. M. Schouls, C. J. Meijer, and P. J. Snijders.** 2002. GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. J. Clin. Microbiol. **40:**779–787.
9. **Zeng, X., F. Kong, H. Wang, A. Darbar, and G. L. Gilbert.** 2006. Simultaneous detection of nine antibiotic resistance-related genes in *Streptococcus agalactiae* using multiplex PCR and reverse line blot hybridization assay. Antimicrob. Agents Chemother. **50:**204–209.
10. **Zhao, Z., F. Kong, and G. L. Gilbert.** 2006. Reverse line blot assay for direct identification of seven *Streptococcus agalactiae* major surface protein antigen genes. Clin. Vaccine Immunol. **13:**145–149.
11. **Zwart, G., E. J. van Hannen, M. P. Kamst-van Agterveld, G. K. Van der, E. S. Lindstrom, J. Van Wichelen, T. Lauridsen, B. C. Crump, S. K. Han, and S. Declerck.** 2003. Rapid screening for freshwater bacterial groups by using reverse line blot hybridization. Appl. Environ. Microbiol. **69:**5875–5883.