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Dot Blot Assay for the Serotyping of Pneumococci

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To simplify the serotyping of *Streptococcus pneumoniae*, a dot blot assay has been developed and compared with the standard quellung reaction in 1,082 isolates. The technique has been demonstrated to be sensitive, specific, easy to perform, and inexpensive. The dot blot assay could be useful when large numbers of pneumococci have to be studied.

Important features of *Streptococcus pneumoniae* and the infections it causes are related to the capsular serotypes. Thus, different serotypes may be associated with different clinical presentations and severities. Serotype distribution varies with age, geographical location, and time. Resistance to beta-lactam antibiotics is restricted to a few serotypes, and virulence for mice is influenced by the capsular type (1, 2). Therefore, typing is an important tool for acquiring a better understanding of all these relationships. Moreover, it is also necessary for a correct formulation of the complex pneumococcal polyvalent vaccines.

Since 1979 our laboratory has typed more than 12,000 pneumococcal isolates received from the microbiological hospital laboratories of Spain. Typing has been carried out by the standard quellung reaction with sera from the Statens Seruminstitut, Copenhagen, Denmark (6). This method is labor-intensive to perform, requires personnel with expertise to interpret the results, and requires a significant amount of serum. Because the number of strains has been increasing year by year, the possibility of an alternative method for typing pneumococci was investigated. We describe here a simple dot blot assay that has been demonstrated to be sensitive and specific, easy to perform, rapid, and inexpensive compared with the standard quellung reaction.

Previous authors have described methods alternative to the quellung reaction for the serotyping of pneumococci, such as capillary precipitin typing (7), counterimmunoelectrophoresis (4, 5), and coagglutination (8). However, these techniques test individual strains or require special equipment, while the dot blot assay allows one to type a great number of strains at the same time (with clear and objective results in most cases), does not require special equipment, and uses a minimal amount of serum. For these reasons this method could be very useful, reducing the man-hours involved when large numbers of pneumococcal isolates need to be studied, as normally occurs in reference laboratories or when surveys of pneumococcal carriers are performed.

The dot blot assay was established with the 15 serogroups, or serotypes (SGTs), prevalent in our country: 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 14, 15, 18, 19, and 23 (3). Five strains of each SGT were cross tested against antisera to the 15 SGTs. Drops of a heavy suspension in saline were spotted along 15 strips of nitrocellulose membranes (Bio-Rad). After 30 min of drying at room temperature (RT) the membranes were blocked for 1 h at RT with 5% skim milk in phosphate-buffered saline, pH 7.2 (PBS). The strips were washed three times with PBS containing 0.05% Tween 20 (PBST), and a 1:20,000 dilution of each antiserum in PBS was added to the corresponding membrane and incubated for 1 h at 37°C on a rocking platform. After being washed three times in PBST, a 1:5,000 dilution of goat antirabbit immunoglobulin G (heavy plus light chains)-peroxidase conjugate (Bio-Rad) in PBS was added and the mixture was incubated for 1 h at RT (different dilutions of sera and conjugate were tested before the mentioned dilutions were chosen). After being washed once in PBST and twice in PBS, the reaction was developed with a 1:3 dilution of a 3-mg/ml ethanol solution of 4 chloronaphthol (Bio-Rad) plus a 1:1,000 dilution of H_2O_2 in PBS. A dark color, often surrounded by a halo, of the spots denoted a positive reaction. These results clearly contrasted with the smaller, weaker spots given by the negative strains. The reactions were stopped with distilled water. With some of the antisera (to SGTs 3, 4, 5, 7, 15, 19, and 23) a cross-reaction with heterologous SGTs occurred, giving intermediate results difficult to interpret. Antibodies against surface antigens common to all pneumococci, such as cell wall polysaccharide (C-polysaccharide), could be responsible for these cross-reactions. Absorption of the sera with the unencapsulated pneumococcal strain R36A rendered them highly

TABLE 1. Comparison of dot blot assay and quellung reaction for typing 1,082 pneumococcal isolates

SGT	No. (%) of strains typed by:	
	Dot blot assay	Quellung reaction
6	143 (13.2)	147 (13.6)
19	135 (12.4)	139 (12.8)
3	109 (10.1)	112 (10.4)
23	101 (9.3)	108 (10.0)
14	92 (8.5)	97 (9.0)
9	65 (6.0)	67 (6.2)
8	49 (4.5)	49 (4.5)
4	36 (3.3)	39 (3.6)
18	35 (3.2)	36 (3.3)
15	30 (2.8)	32 (3.0)
1	28 (2.6)	29 (2.7)
7	20 (1.8)	20 (1.8)
11	20 (1.8)	20 (1.8)
12	15 (1.4)	16 (1.5)
5	12 (1.1)	14 (1.3)
Others[a]		117 (10.8)
NT[b]		40 (3.7)
Total	890 (82)	1,082 (100)

[a] Other SGTs: 2, 10, 13, 16, 17, 20, 21, 22, 24, 27, 28, 31, 32, 33, 34, 35, 37, 38, and 42.
[b] NT, nontypeable (noncapsulated) strains.

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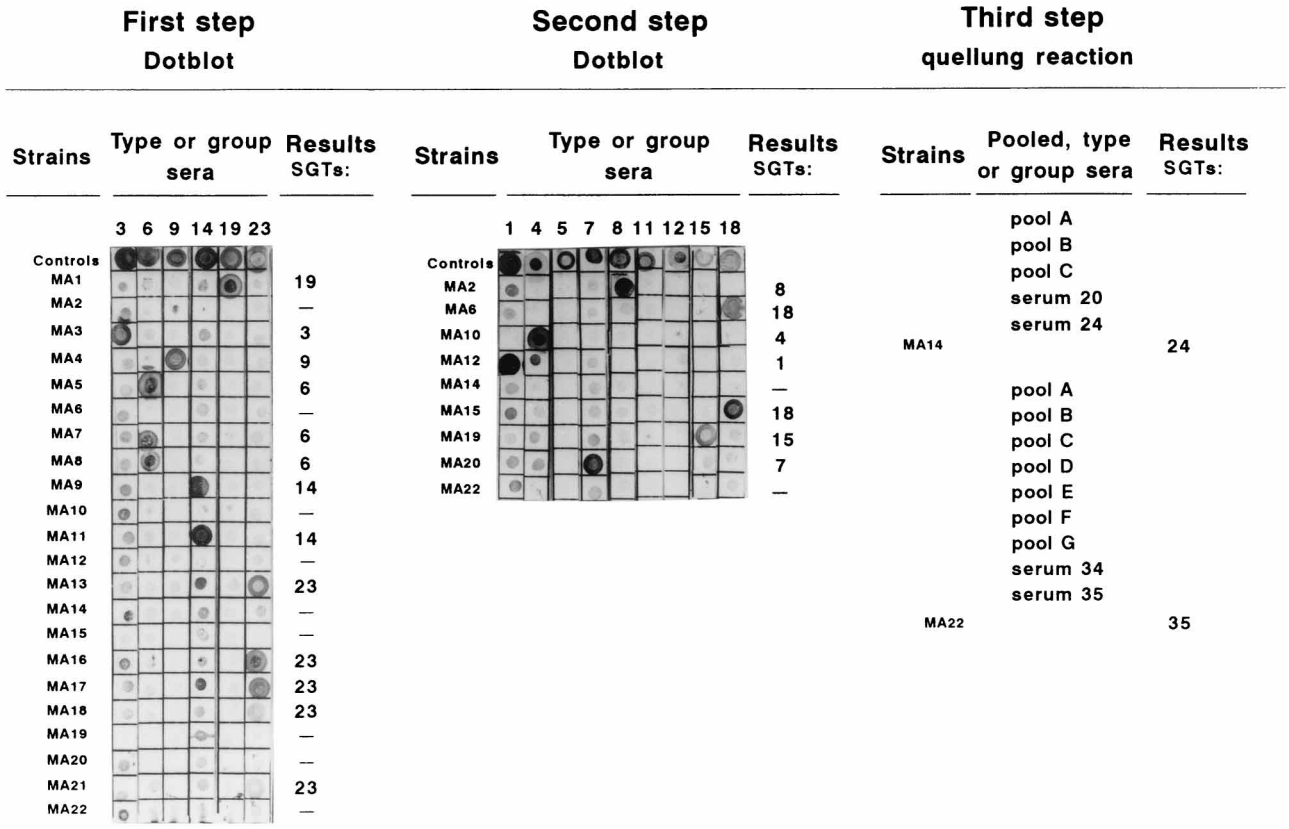

FIG. 1. Scheme for the routine typing of pneumococci.

specific. We observed that clearer dot blot results were obtained when old suspensions, stored for a week at 4°C, were used. This probably could be explained by the release of the polysaccharide caused by autolysis.

In view of the good results obtained, the evaluation of the dot blot assay as a routine typing system was carried out. During the first 6 months of 1996, 1,082 pneumococcal isolates received in our laboratory and typed by the standard quellung reaction were blind typed also by dot blot assay with antisera to the 15 prevalent SGTs, and the results of the two methods were compared (Table 1). In dot blot typing, a pneumococcus for which the antiserum was specific was tested in each strip as a positive control and results were interpreted by comparison with the controls. All the positive results obtained by dot blot assay were confirmed by the quellung reaction (100% specificity), so there were no false positives. A total of 890 of the 1,082 strains studied (82%) could be correctly typed by dot blot assay with the 15 antisera, but the technique failed to type 35 (3.8%) pneumococci belonging to the SGTs included in the dot blot assay (96% sensitivity). A total of 117 pneumococci of 19 different SGTs not included in the dot blot assay were typed only by the quellung reaction. Finally, 40 (3.7%) isolates were considered nontypeable, since it was not possible to detect the presence of capsule on them.

In order to simplify the typing work and reduce cost and time, the following strategy is now used routinely in the laboratory, based on the SGT distribution of the pneumococci isolated in Spain (3). The typing scheme is done in three steps. First, we test all pneumococci against antisera to SGTs 3, 6, 9, 14, 19, and 23, since about 60% of the total strains belong to them. Those isolates that cannot be typed by the first round are tested in a second step against antisera to the other nine prevalent SGTs, 1, 4, 5, 7, 8, 11, 12, 15, and 18, which account for another 20% of the strains. Finally, the strains that are negative or that give equivocal results by dot blot assay with these 15 antisera are typed directly by the quellung reaction. As an example, in Fig. 1, strains MA18 and MA21, which gave a weak reaction with antiserum to SGT 23, and strains MA14 and MA22, which did not react with the antisera to the 15 more frequent SGTs, needed to be typed by the quellung reaction.

We have used only 15 antisera, but preliminary studies suggest that the same results could be obtained with other SGT antisera. However, unclear results have been obtained with factor sera. In our hands, and because of the reduced number of the other SGTs, it is easier and more economical to test them directly by the quellung reaction; however, this situation can be different in other countries or in the case of a change in the distribution of SGTs.

Although the quellung reaction must be maintained as the reference technique for typing pneumococci, the dot blot assay is a sensitive, specific, rapid, and economical system that has permitted a reduction in the number of strains that need to be tested by the quellung reaction to only 20% of all pneumococci received in our laboratory.

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