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Letters to the Editor

Serotype-Specific Typing Antisera for Pneumococcal Serogroup 6 Serotypes 6A, 6B, and 6C[▽]

Streptococcus pneumoniae strains can express one of at least 92 capsular serotypes. To our knowledge, our laboratory is one of two that maintain antisera for resolution of the first 90 discovered pneumococcal serotypes (4; unpublished data). Recently, the evaluation of serogroup 6 isolates using monoclonal antibodies led to the discovery of the 91st serotype, 6C (7), which has become the prevalent invasive serogroup 6 serotype within the United States (2, 9). The pneumococcal 7-valent conjugate vaccine (PCV7) does not protect against it, and it is not included within the newly licensed 13-valent conjugate vaccine. In addition, serotype 6C carriage may also be common within PCV7-vaccinated populations (3). Prior to the discovery of serotype 6C, serotypes 6A and 6B were the 2 known serogroup 6 serotypes. CDC antisera for quellung-based resolution of serotypes 6A and 6B are designated as Danish factors 6b and 6c (DF-6b and DF-6c), respectively. Our original DF-6b antiserum was positive for both 6A and 6C serotypes, preventing their resolution (Table 1).

We produced DF-6d antiserum specific to serotype 6C and DF-6b antiserum specific to 6A as follows. New Zealand White female rabbits were inoculated with *S. pneumoniae* serotypes 6C or 6A formalin-fixed whole cells. The inocula consisted of serotype 6C or 6A cell suspensions (10^8 to 10^9 cells per ml in phosphate-buffered saline), which were administered intravenously via the marginal ear vein (Table 2). Blood was drawn after week 4 of dosing, and serum was evaluated for antibody titer to serotype 6C or 6A and cross-reactivity to serotypes 6A, 6B, and 6C by the quellung reaction. Cross-reactivity was removed from DF-6d by absorbing the serum with formalin-fixed cells of serotypes 6A and 6B. DF-6b antiserum was absorbed with formalin-fixed cells of serotypes 6B and 6C. The resulting DF-6d antiserum was specific for serotype 6C only, and DF-6b was specific to 6A only (Table 1).

The specificity of DF-6b, DF-6c, and DF-6d antisera was evaluated with stock strains and 159 clinical isolates, each representing one of the serotypes 6A, 6B, or 6C. For all strains, Neufeld quellung results were compared to PCR testing specific for the serotype 6C $wicN_{6C}$ locus (2, 7, 8) with no inconsistencies observed.

We have replaced our previous methods with these procedures for producing specific factor sera against serotypes 6A and 6C. We have now learned that Statens Serum Institute (SSI) has also recently produced factor 6d against serotype 6C (5). Our DF-6b, unlike the SSI factor 6b, is now serotype 6A specific and does not cross-react against serotype 6C.

Serotype 6C-specific antiserum will be useful for laboratories which utilize quellung testing. Recently a fourth serogroup 6 serotype, serotype 6D, has been discovered in nature (1, 6) that is recognizable by a positive quellung test for serotype 6B combined with a positive PCR test for $wciN_{6C}$. Despite extensive screening, we have not yet encountered this serotype (2). We do not yet know if our DF-6d antiserum would cross-react with serotype 6D. Consequently we screen all quellung test 6B strains by PCR for the presence of $wciN_{6C}$ (2, 8).

TABLE 1. DF-6b, DF-6c, and DF-6d quellung results

Serotype	Reaction with Danish factor antiserum:			
	Original DF-6b[a]	New DF-6b[b]	DF-6c[c]	DF-6d[d]
6A	+	+	−	−
6B	−	−	+	−
6C	+	−	−	+

[a] Original serotype 6A quellung serum (absorbed against serotype 6B).
[b] New serotype 6A quellung serum (absorbed against serotypes 6B and 6C).
[c] Original serotype 6B quellung serum (absorbed against serotype 6A).
[d] New serotype 6C quellung serum (absorbed against serotypes 6A and 6B).

TABLE 2. Dosing schedule for preparation of antisera

Wk	Dose (ml) on[a]:		
	Day 1	Day 2	Day 3
1	0.1	0.2	0.2
2	0.2	0.3	0.4
3	0.4	0.5	0.5

[a] After week 3, the dose was 0.5 ml for the duration of the study.

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