JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2010, p. 3044–3045 Vol. 48, No. 8
0095-1137/10/$12.00 doi:10.1128/JCM.00839-10

Cross-Reactivity of Current Serogroup 6 Factor Sera from Statens Serum Institut with the Recently Described Pneumococcal Serotype 6D▽

A recent study by Jacobs et al. (3) examined the specificity of a newly introduced pneumococcal capsule-specific antiserum (factor serum 6d) manufactured by Statens Serum Institut (SSI) in Denmark. The factor serum 6d was designed to be specific for serotype 6C, a newly described pneumococcal serotype (5), and was to complement factor sera 6b and 6c, which are designed to be specific for pneumococcal serotypes 6A and 6B, respectively. While Jacobs et al. confirmed the designed specificity of the factor serum 6d for serotype 6C using the standard Quellung reaction, the study did not examine the specificity of the factor serum for the novel 6D serotype, which was previously predicted (2) but was only recently found in nature (1, 4). We have therefore investigated the specificity of the currently available serogroup 6 factor sera with serotype 6D using the standard Quellung reaction, an agglutination assay, and a flow cytometric assay.

The three SSI serogroup 6 rabbit factor sera (6b [lot L6b22A1], 6c [lot H6c11E1], and 6d [lot L6d11C1]) were purchased from Mira Vista Diagnostics in March of 2010. The 6b factor serum was manufactured using the protocol revised in January 2009 and was absorbed with serotype 6C according to SSI (personal communication). Initially, we tested the factor sera using the Quellung reaction and four pneumococcal strains, TIGR6A, -B, -C, and -D, which are artificially created pneumococcal strains in the TIGR4 background that express pneumococcal capsule types 6A, 6B, 6C, and 6D, respectively (2). Using the Quellung reaction, we found that factor sera 6b, 6c, and 6d reacted with serotypes 6A, 6B, and 6C, respectively, while both factor sera 6c and 6d also reacted with serotype 6D (Table 1). To further investigate the seroreactivity of these factor sera, we tested the sera with flow cytometry and agglutination assays. The agglutination assay was independently performed by three individuals who were blinded to the identities of the three factor sera and eight bacterial strains (Table 2). The bacterial strains consisted of four clinical isolates representing the four serotypes and the TIGR6A, -B, -C, and -D strains. Again, we found that factor sera 6b, 6c, and 6d reacted with serotypes 6A, 6B, and 6C, respectively (Table 2), although one operator scored the reaction of TIGR6C with factor serum 6c as positive. As a result of this discrepancy, 12 additional clinical 6C strains were tested with factor serum 6c. Eleven of these were scored as negative, and one was scored as weakly positive. We also found that factor sera 6c and 6d reacted with serotype 6D, although there was some disagreement with the TIGR6D strain and factor serum 6c. Overall, the agglutination test results closely resembled the Quellung reaction results, and serotype 6D seems to react positively with both factor sera 6c and 6d.

TABLE 1. Specificity of the serogroup 6 factor sera in the Quellung test

Strain	Reaction[a] with factor serum:		
	6b	6c	6d
TIGR6A	+	−	−
TIGR6B	−	+	−
TIGR6C	−	−	+
TIGR6D	−	+	+

[a] +, positive reaction; −, negative reaction.

TABLE 2. Specificity of serogroup 6 factor sera in the agglutination test

Strain	Reaction[a] with factor serum:		
	6b	6c	6d
MNZ13 (6A)	+	−	−
TIGR6A	+	−	−
MNZ31 (6B)	−	+	−
TIGR6B	−	+	−
MNZ16 (6C)	−	−	+
TIGR6C	−	−[b]	+
MNZ21 (6D)	−	+	+
TIGR6D	−	+[c]	+

[a] +, positive reaction; −, negative reaction.
[b] Two operators gave a negative result, and one recorded a positive result.
[c] Two operators gave a positive result, and one recorded a negative result.

Flow cytometry was performed as previously described (1), using the factor sera at a 1:50 dilution and goat anti-rabbit R–phycoerythrin conjugate (Southern Biotech). We considered staining under 30 fluorescence units to be negative, and there is moderate staining of some factor sera for multiple serotypes. However, the moderate staining may not be meaningful since these factor sera are not meant for use in flow cytometry. The more meaningful results may be the strong staining (more than 300 fluorescence units) produced by the factor sera (Fig. 1). With strong staining as the selection criterion, the factor sera bound to the expected serotypes: factor serum 6d bound to 6C, and factor sera 6c and 6d reacted with

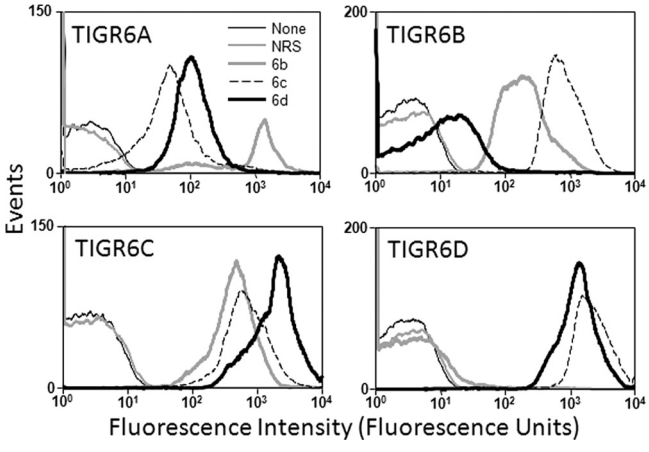

FIG. 1. Results of flow cytometry performed with pneumococcal strains TIGR6A, -B, -C, and -D. Controls included are None (no primary antibody) and NRS (normal, unimmunized rabbit serum). 6b, 6c, and 6d represent factor sera 6b, 6c, and 6d, respectively.

serotype 6D, similar to the Quellung reaction results. Taken together, our results confirm and extend the findings of Jacobs et al. by showing that the currently available factor sera 6c and 6d may cross-react with serotype 6D. Furthermore, their cross-reaction may be useful in the serological identification of serotype 6D. However, this cross-reaction with 6D may vary among reagent lots since the currently available factor sera may not be tested for cross-reaction with serotype 6D.

This work was supported by NIH funding (AI-031473) to M.H.N.

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▽ Published ahead of print on 23 June 2010.