

Assignment of Additional Anticapsular Antibody Concentrations to the *Neisseria meningitidis* Group A, C, Y, and W-135 Meningococcal Standard Reference Serum CDC1992

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We assigned additional enzyme-linked immunosorbent assay antibody concentrations (immunoglobulin G [IgG], IgM, and IgA, and total) to the *Neisseria meningitidis* standard reference serum CDC1992 for groups Y and W-135 to 12 Centers for Disease Control and Prevention quality control sera. These assignments will supplement previous assignments and will aid in the evaluation of present and developing vaccines.

Meningococcal vaccine evaluation requires the use of quantitative and functional antibody determinations by enzyme-linked immunosorbent assay (ELISA) and serum bactericidal activity assay, respectively. Both types of assays require standard reference sera and quality control sera for antibody measurements and comparisons.

An ELISA has been standardized for quantification of total (total = summation of IgG + IgM + IgA $\mu\text{g/ml}$) and class-specific (IgG, IgM, and IgA) meningococcal group A and C anticapsular antibodies (1, 3). A standard reference serum (CDC1992) was prepared, and total and class-specific anticapsular antibody concentrations were previously assigned for groups A and C (4). However, no assignments were made for groups Y and W-135. Anticapsular antibody concentrations were determined for a panel of twelve Centers for Disease Control and Prevention (CDC) adult quality control sera from donors vaccinated with Menomune (Connaught Laboratories, Inc., Swiftwater, Pa.) (5). Total and class-specific anticapsular antibody concentrations for meningococcal groups Y and W-135 were assigned to CDC1992 for this report, and antibody concentrations for groups A, C, Y, and W-135 are also reported for twelve CDC quality control sera.

The standardized ELISA was used to determine the group Y and W-135 anticapsular antibody concentrations as described previously by Carlone et al. (1) and Gheesling et al. (3). All polysaccharides were prepared by Aventis Pasteur (Swiftwater, Pa.). Group A and C polysaccharides, CDC1992, and methylated human serum albumin (mHSA) are available on request from the National Institute for Biological Standards and Control in the United Kingdom. Group Y and W-135 polysaccharides were kindly provided by Aventis Pasteur. Assignments for groups A and C were determined by using heterologous assays as reported previously by Holder et al. (4). These assignments were in turn used in additional heterologous ELISA to assign antibody concentrations for group Y and W-135 in CDC1992.

This method of cross-standardization has been reported by Concepcion and Frasch (2). Mean antibody concentrations for IgG, IgM, and IgA, which are presented in Table 1, were determined for groups Y and W-135 by using horseradish peroxidase-labeled mouse anti-human monoclonal antibody conjugates. The IgG(HP6043) conjugate was prepared by the Hybridoma Reagent Laboratory (Baltimore, Md.), and the IgM(HP6083) and IgA(HP6123) conjugates were produced at the CDC. These CDC clones are available commercially and can be conjugated with enzymes by using standard methods. The detection substrate was TMB (3,3',5,5'-tetramethylbenzidine)–0.01% hydrogen peroxide (Kirkegaard & Perry Laboratories, Gaithersburg, Md.). Since the previously reported ELISA used alkaline phosphatase-labeled conjugates and other preparations of polysaccharides and mHSA, appropriate crossover studies were done with horseradish peroxidase-labeled conjugates and National Institute for Biological Standards and Control preparations of polysaccharides and mHSA. A minimum of three independent assays of each quality control serum were performed. The results showed no statistically significant differences ($P < 0.05$) for any comparisons (data not shown). The quality control sera were assigned anticapsular antibody concentrations for groups Y and W-135 after the antibody concentrations were assigned to CDC1992. Data analysis for ELISA antibody concentrations was done by using a four-parameter logistic log curve-fitting technique (6, 7). All antibody concentrations were calculated within the working range of the standard curve.

The antibody concentrations for groups A, C, Y, and W-135 for the standard reference serum CDC1992 and for twelve CDC quality control sera are shown in Table 1.

In summary, we have assigned total and class-specific antibody concentrations for groups Y and W-135 to the meningococcal standard reference serum CDC1992 by using an ELISA protocol previously standardized for groups A and C. Antibody concentrations are also reported for a panel of CDC quality control sera. The anticapsular antibody concentrations for CDC1992 and the twelve quality control sera reported here will aid in the inter- and intralaboratory evaluation of present and developing meningococcal vaccines.

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TABLE 1. ELISA anticapsular antibody concentrations in CDC1992 and 12 CDC quality control sera for meningococcal groups A, C, Y, and W-135

Serum sample no.	Ab concentration ($\mu\text{g/ml}$) for meningococcal capsular polysaccharide [mean (SE)] ^a															
	Group A ^b				Group C ^b				Group Y				Group W-135			
	IgG	IgM	IgA	Total ^c	IgG	IgM	IgA	Total ^c	IgG	IgM	IgA	Total ^c	IgG	IgM	IgA	Total ^c
CDC1992	91.8 (8.7)	23.9 (1.4)	20.1 (1.2)	135.8	24.1 (1.1)	2.0 (0.1)	5.9 (0.2)	32.0	31.8 (2.4)	2.9 (0.2)	3.7 (0.4)	38.4	16.2 (1.0)	1.4 (0.1)	2.5 (0.2)	20.1
900215	515.5 (24.6)	60.6 (0.8)	34.3 (0.9)	610.4	11.9 (0.4)	16.7 (1.4)	7.7 (0.6)	36.3	35.1 (3.7)	2.1 (0.2)	9.7 (0.5)	46.9	25.9 (2.6)	3.2 (0.2)	10.0 (1.3)	39.1
900242	17.2 (0.6)	35.0 (0.8)	50.2 (1.4)	102.4	119.9 (12.1)	24.5 (1.1)	101.9 (8.5)	246.3	48.3 (2.7)	2.2 (0.3)	15.0 (0.9)	65.5	4.5 (0.4)	5.0 (0.5)	4.7 (0.5)	14.2
900243	24.5 (0.2)	77.3 (1.4)	9.8 (0.2)	111.6	32.8 (1.8)	9.5 (1.1)	9.6 (0.6)	51.9	134.1 (9.3)	5.2 (0.6)	6.2 (0.5)	145.5	6.7 (1.1)	4.1 (0.3)	2.7 (0.2)	13.5
900268	39.6 (2.0)	18.6 (0.5)	15.2 (0.3)	73.4	38.0 (0.9)	2.3 (0.2)	21.0 (1.8)	61.3	21.9 (2.1)	2.8 (0.3)	5.4 (0.2)	30.1	8.7 (0.7)	2.3 (0.1)	1.9 (0.2)	12.9
900274	143.6 (9.4)	24.4 (2.7)	7.4 (0.7)	175.4	15.4 (1.3)	11.1 (1.0)	37.9 (4.1)	64.4	4.2 (0.7)	1.9 (0.2)	2.3 (0.2)	8.4	1.1 (0.1)	1.3 (0.1)	0.7 (0.1)	3.1
900305	20.4 (0.8)	25.7 (1.6)	4.5 (0.5)	50.5	66.6 (0.2)	6.7 (0.6)	14.4 (1.0)	87.7	8.4 (1.3)	10.7 (1.4)	4.6 (0.3)	23.7	2.2 (0.2)	13.9 (0.7)	1.2 (0.1)	17.3
900306	1.5 (0.1)	1.5 (0.1)	0.6 (0.1)	3.6	74.1 (6.2)	0.3 (0.1)	0.7 (0.1)	75.1	279.2 (22.4)	0.3 (0.0)	7.1 (0.4)	286.6	122.1 (10.5)	0.1 (0.0)	1.9 (0.2)	124.1
900323	20.0 (1.3)	10.0 (1.1)	14.2 (1.1)	44.3	76.4 (8.4)	6.6 (0.7)	12.0 (0.6)	95.0	28.0 (2.0)	2.5 (0.3)	13.3 (0.5)	43.8	56.8 (5.2)	1.0 (0.0)	6.0 (0.4)	63.8
900329	77.7 (4.1)	1.7 (0.1)	7.5 (0.8)	86.9	24.8 (3.3)	1.1 (0.2)	7.2 (0.8)	33.1	111.1 (6.8)	2.3 (0.2)	29.1 (2.2)	142.5	30.7 (2.7)	1.1 (0.1)	9.8 (1.3)	41.6
900385	40.2 (3.5)	3.6 (0.2)	12.8 (1.0)	56.6	42.0 (4.8)	1.6 (0.1)	22.7 (0.7)	66.3	7.3 (0.6)	0.2 (0.0)	2.3 (0.2)	9.8	10.5 (1.0)	1.6 (0.1)	10.4 (1.3)	22.5
900414	49.8 (3.9)	12.6 (1.0)	5.5 (0.8)	67.9	26.5 (5.3)	1.0 (0.1)	1.7 (0.1)	29.2	45.9 (4.9)	1.9 (0.2)	7.2 (0.3)	55.0	42.6 (4.1)	1.7 (0.1)	6.1 (0.4)	50.4
900415	7.0 (0.6)	11.1 (1.4)	2.4 (0.2)	20.5	2.9 (0.2)	1.2 (0.2)	1.0 (0.1)	5.1	45.7 (3.2)	0.2 (0.0)	1.0 (0.1)	46.9	8.9 (0.9)	0.2 (0.0)	0.5 (0.1)	9.6

^a Mean (SE), mean concentration \pm standard error for at least three independent assays per measurement. Ab, antibody.

^b Concentrations for serogroups A and C were previously published by Holder et al. (4) for the following sera: CDC1992, 900215, 900242, 900268, 900274, 900306, and 900414.

^c Total Ab concentration (total = summation of IgG + IgM + IgA) determined by summation of mean class-specific Ab concentrations.

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