Supporting Information

Donnelly et al. 10.1073/pnas.1013758107

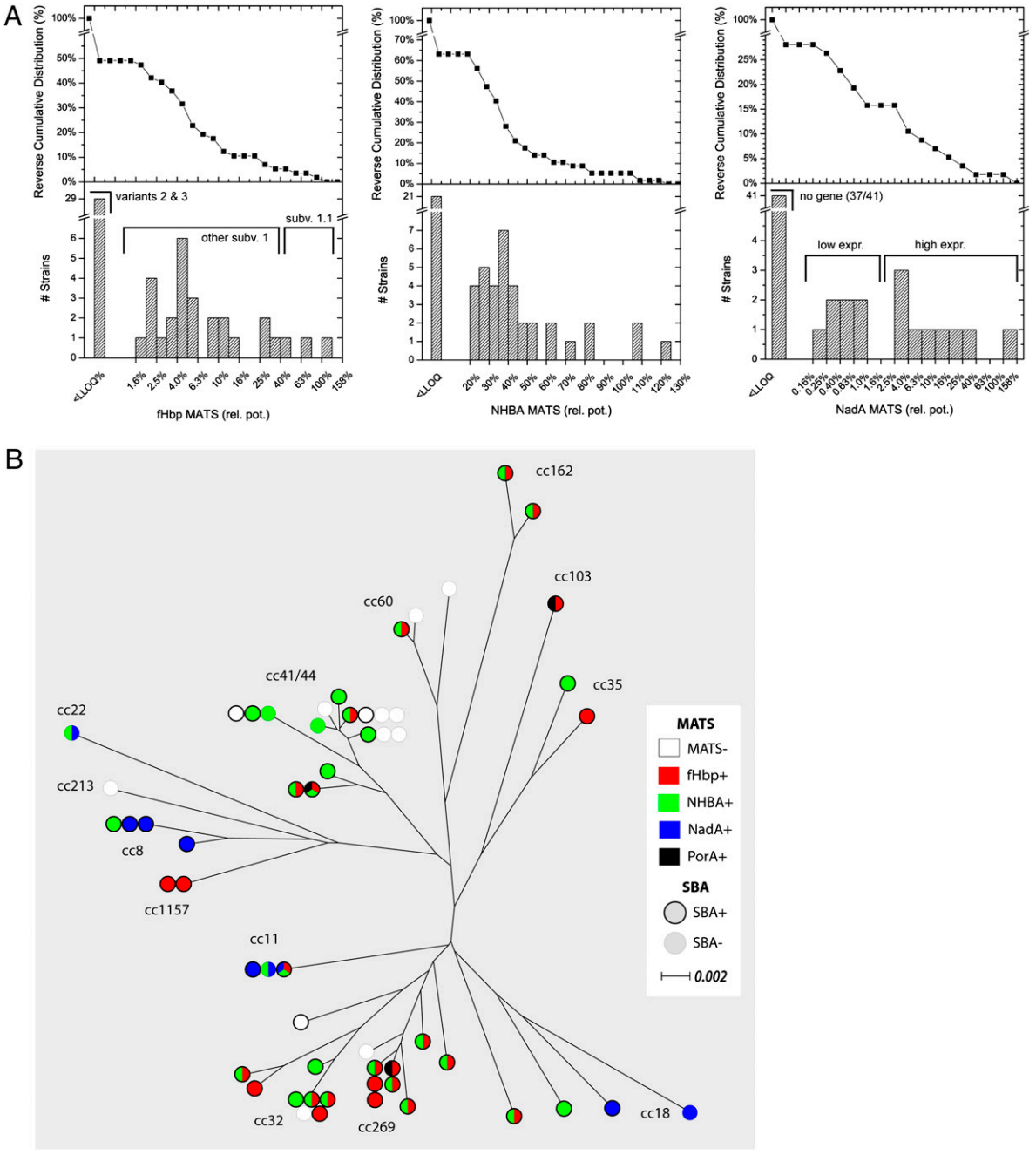

Fig. S1. MATS relative potencies and SBA results with pooled infant sera for the subset of 57 MenB strains. (*A*) Frequency distribution of antigen relative potency (RP) of NHBA, NadA, and fHbp antigens in 57 selected serogroup B strains. (*Upper*) Reverse cumulative distributions of the proportion of strains with antigen RP greater than the values indicated on the *x* axis. (*Lower*) Histograms of frequency distribution of strains with different values of antigen RP. As in Fig. 2*A*, antigen RP is shown of different strains for fHbp. Genetic variants (major variants denoted by 1, 2, or 3 and subvariants denoted by decimals) of fHbp are indicated by brackets. As in Fig. 2*B*, antigen relative potencies are shown of different strains for NHBA. As in Fig. 2*C*, antigen relative potencies are shown of different strains for NadA. Brackets denote strains with low and high expression of NadA. Eighty-eight percent of the strains not quantifiable by the test are PCR negative for the NadA gene. RP, relative potency for each antigen determined by MATS in comparison with the reference strains H44/76 (fHbp), 5/99 (NadA), and NGH38 (NHBA). LLOQ, lower limit of quantitation, lowest antigen RP values with between-assay CV ≤25%. (*B*) MLST-based phylogenetic reconstruction of the 124 strains that were MATS typed in this study. Color coding shows antigens at or above the PBT. Strains killed in the SBA with postimmune serum from adult vaccinees are coded by a thick black border. Major clonal complexes are indicated with the founder ST. The phylogenetic tree was obtained with the Neighbor program from the PHYLIP package, with branch lengths computed from the Kimura two-parameter distances.

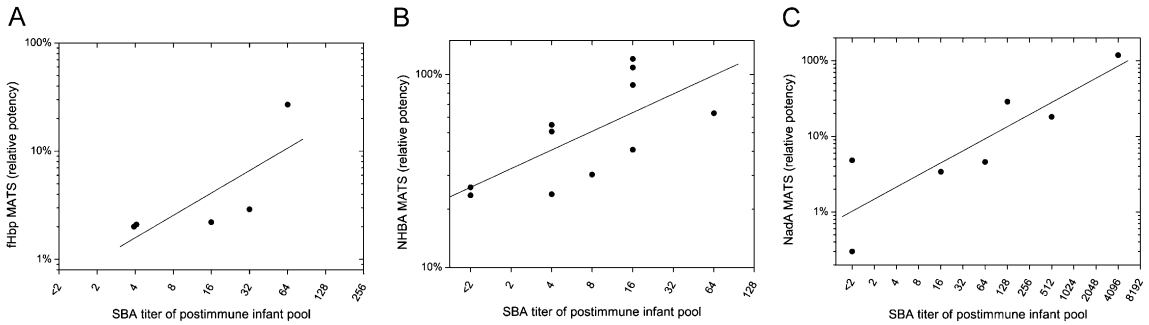

Fig. S2. MATS relative potency vs. SBA titer in pooled postbooster sera from children immunized at 2, 4, and 6 mo of age and boosted at 12 mo. Selected subsets of strains (denoted by solid circles) with fHbp (n = 5) (*A*), NHBA (n = 11) (*B*), or NadA (n = 7) (*C*) above the lower limit of quantitation (LLOQ) and all others below the LLOQ were evaluated for SBA activity and MATS relative potency. The regression line (–) was obtained from log-transformed data by the least-squares method; Pearson's product-moment correlation coefficient r^2 = 0.58, 0.47, and 0.75 for fHbp, NHBA, and NadA, respectively. Spearman's non-parametric rank correlations coefficients were 0.97 (P = 0.005), 0.75 (P = 0.008), and 0.81 (P = 0.027) for fHbp, NHBA, and NadA, respectively.

Table S1. ***Neisseria meningitidis*** **group B strains used**

	Strain characterization						
	CC	ST	Year	Country	Serotype/sero-subtype	fHbp variant		*nadA* gene
Strain						Old	New	
96217	8	153	1996	CAN	B:2b:P1.5,2	2.1	2.p0016	+
5/99	8	1349	1999	N	B:2b:P1.5,2	2.8	2.p0023	+
M01-0240988	213	213	2001	U.K.	B:1:P1.22,14	3.3	3.p0030	+
8047	11	11	1978	USA	B:2:P1.2	2.18	2.p0059	+
B3937	18	New	1995	D	B:22:P1.16	2.2	2.p0017	+
M10525	60	6148	2003	USA	B:ND:P1.5-1,2	2.8	2.p0023	(–)
M10994	41/44	44	2003	USA	B:ND:P1.21,16	2.4	2.p0019	(–)
M1239	41/44	437	1994	USA	B:14:P1.23,14	3.1	3.p0028	(–)
M14815	32	32	2006	USA	B:ND:P1.7,16	3.3	3.p0030	+
M3153	41/44	5906	1996	USA	B:4,7:P1.7-1,1	2.4	2.p0019	(–)
M3369	Ua	1576	1997	USA	B:10:P1.19,15	3.4	3.p0031	(–)
M4458	22	new	1998	USA	B:NT:P1.3	2.10	2.p0025	+
H44/76	32	32	1976	N	B:15:P1.7,16	1.1	1.p0001	(–)
NMB	8	1380	1968	USA	B:2b:P1.5,2	2.1	2.p0016	+
M14296	162	162	2005	USA	B:ND:P1.22,14	1.p0180	1.p0180	(–)
M16019	32	32	2007	USA	B:ND:P1.7,16	1.1	1.p0001	+
M15563	162	162	2006	USA	B:ND:P1.22,14	1.9-3	1.p0013	(–)
M01-0240364	11	11	2001	U.K.	B:2a:P1.5,2	3.4	3.p0031	+
M01-0240660	269	1049	2001	U.K.	B:NT:P1.19,15	1.11	1.p0015	(–)
ISS1104	32	32	2000	I	B:15:P1.7,16	1.1	1.p0001	+
M10574	32	803	2003	USA	B:ND:P1.7-2,13-1	3.15	3.p0061	+
M10837	41/44	409	2003	USA	B:ND:P1.18-1,34-2	2.4	2.p0019	(–)
M14459	Ua	2048	2005	USA	B:ND:P1.22,9	1.p0180	1.p0180	(–)
M18483	103	103	2008	USA	B:ND:P1.7-2,4	1.9-2	1.p0012	(–)
NZ98/254	41/44	42	1998	NZ	B:4:P1.7-2,4	1.10	1.p0014	(–)
M11048	60	60	2003	USA	B:ND:P1.5-1,2-2	1.9-3	1.p0013	(–)
M18632	1157	1157	2008	USA	B:ND:P1.5-1,9	1.9-3	1.p0013	+
M4030	Ua	178	1993	USA	B:17:P1.19,15	1.9-2	1.p0012	(–)
M01-0240500	269	269	2001	U.K.	B:NT:P1.7,4	1.11	1.p0015	(–)
M01-0240993	11	11	2001	U.K.	B:2a:P1.5-1,10-8	1.9-1	1.p0011	+
LNP19324	32	33	1998	F	B:2b:P1.5,2	1.14	1.p0041	+
M13202	41/44	1194	2004	USA	B:ND:P1.18-1,3	1.4	1.p0004	(–)
M14933	32	32	2006	USA	B:ND:P1.22,14	3.15	3.p0061	+
M15295	32	34	2006	USA	B:ND:P1.19,15	1.p0196	1.p0196	+
M16686	41/44	2487	2007	USA	B:ND:P1.7-1,1	1.9-3	1.p0013	(–)
M18133	41/44	41	2008	USA	B:ND:P1.22,14	1.4	1.p0004	(–)
M18339	1157	1157	2008	USA	B:ND:P1.22,14-6	1.9-3	1.p0013	+
M01-0240185	11	11	2001	U.K.	B:2a:P1.5,10	1.9	1.p0010	(–)
M01-0240889	269	1214	2001	U.K.	B:NT:P1.5,10	1.11	1.p0015	(–)
M10713	41/44	136	2003	USA	B:ND:P1.17,16-3	2.9	2.p0024	(–)
961–5945	8	153	1996	AUS	B:2b:P1.21,16	2.1	2.p0016	+
ISS1026	41/44	44	2000	I	B:4:P1.13	1.10	1.p0014	(–)
LNP17094	8	153	1999	F	B:2b:P1.10	2.1	2.p0016	+
M10566	41/44	437	2003	USA	B.ND:P1.22-1,14	2.4	2.p0019	(–)
M12886	Ua	6147	2004	USA	B:ND:P1.22-15,28-2	1.4	1.p0004	(–)
M13032	162	753	2004	USA	B:ND:P1.22,14	1.23	1.p0065	(–)
M14879	1157	1157	2006	USA	B:ND:P1.22,14-6	1.9-3	1.p0013	+
M14882	41/44	44	2006	USA	B:ND:P1.7-1,1	2.4	2.p0019	(–)
M2937	35	new	1996	USA	B:4,7:P1.23,14	1.7	1.p0007	(–)
M3812	60	60	1997	USA	B:NT:P1.5-1,10-4	1.9-2	1.p0012	(–)
M4407	41/44	new	1996	USA	B:NT:P1.15	2.4	2.p0019	(–)
NGH38	Ua	36	1988	N	B:NT:P1.3	2.9	2.p0024	(–)
NM117	269	1195	1998	U.K.	B:21:P1.9	1.11	1.p0015	(–)
M01-0240101	269	1049	2001	U.K.	B:NT:P1.19,15	1.11	1.p0015	(–)
M15978	60	60	2007	USA	B:ND:P1.5,2	1.9-3	1.p0013	(–)
M01-0240443	269	269	2001	U.K.	B:NT:P1.5,2	1.11	1.p0015	(–)
M12898	35	457	2004	USA	B:ND:P1.5-1,2-2	2.1	2.p0016	(–)
M16405	41/44	136	2007	USA	B:ND:P1.7-2,13-1	2.p0218	2.p0218	(–)
M11822	41/44	2331	2004	USA	B:ND:P1.22-1,14	2.4	2.p0019	(–)
M12425	41/44	44	2004	USA	B:ND:P1.7-1,1	1.33	1.p0083	(–)

Table S1. Cont.

	Strain characterization						
	CC	ST	Year	Country	Serotype/sero-subtype	fHbp variant	*nadA* gene
M15009	41/44	136	2006	USA	B:ND:P1.17,16-3	2.9	2.p0024	(–)
M17632	41/44	437	2008	USA	B:ND:P1.22-1,14	2.4	2.p0019	(–)
U.K.200	269	275	2001	U.K.	B:NT:P1.22,9	1.9-3	1.p0013	(–)
03S-0658	32	1364	—	USA	B:3:P1.7-2,13-1	2.8	2.p0023	+
03S-0673	32	1364	—	USA	B:3:P1.7-2,13-1	2.8	2.p0023	+
1000	18	20	1988	RUS	B:NT:P1.5,10-4	2.10	2.p0025	(–)
2996	8	540	1975	U.K.	B:2b:P1.5,2	2.1	2.p0016	+
67/00	41/44	1127	2000	N	B:4,7:NST	1.10	1.p0014	(–)
72/00	32	1346	2000	N	B:15:P1.7,13	1.1	1.p0001	+
95N477	11	475	1995	AUS	B:2a:P1.2	2.7	2.p0022	(–)
972–0319	41/44	158	1997	AUS	B:NT:P1.4	1.10	1.p0014	(–)
BZ198	41/44	41	1986	NL	B:NT:P1.7-2,4	1.10	1.p0014	(–)
BZ232	37	38	1964	NL	B:NT:P1.2	2.9	2.p0024	(–)
CU385	32	33	1980	C	B:4:P1.15	1.1	1.p0001	+
D8221	231	2744	2002	D	B:NT:P1.5,10-1	2.9	2.p0024	(–)
ISS1102	41/44	2916	2000	I	B:15:P1.4	1.10	1.p0014	(–)
ISS1106	41/44	42	2000	I	B:4:P1.4	1.10	1.p0014	(–)
ISS749	41/44	1127	1996	I	B:14:P1.13	1.10	1.p0014	(–)
ISS832	32	32	1997	I	B:15:P1.7,16-6	1.1	1.p0001	+
LNP20404	32	800	2003	F	B:14:P1.7,16	1.1	1.p0001	+
M0579	41/44	43	1993	USA	B:ND:P1.5,2	2.5	2.p0020	(–)
M10549	35	457	2003	USA	B:ND:P1.22-1,14	2.1	2.p0016	(–)
M11003	41/44	5097	2003	USA	B.ND:P1.7-2,4	1.4	1.p0004	(–)
M11053	269	275	2003	USA	B:ND:P1.22,9	2.4	2.p0019	(–)
M11095	41/44	136	2003	USA	B:ND:P1.18-1,3	2.9	2.p0024	(–)
M11204	41/44	5109	2003	USA	B:ND:P1.7-1,1	2.4	2.p0019	(–)
M11295	32	32	2003	USA	B:ND:P1.7,16	1.1	1.p0001	+
M11906	41/44	44	2004	USA	B:ND:P1.7-1,1	1.33	1.p0083	(–)
M12502	162	162	2004	USA	B:ND:P1.22,14	1.9-3	1.p0013	(–)
M12550	41/44	4682	2004	USA	B:ND:P1.22-1,14	1.9-3	1.p0013	(–)
M12566	41/44	5111	2004	USA	B:ND:P1.21,16	2.4	2.p0019	(–)
M13203	41/44	44	2005	USA	B:ND:P1.7-1,1	2.4	2.p0019	(–)
M1390	41/44	41	1995	USA	B:15:B1.7,4	1.10	1.p0014	(–)
M14549	103	6063	2005	USA	B:ND:P1.17,16-3	2.10	2.p0025	(–)
M14613	162	162	2005	USA	B:ND:P1.22,14	3.4	3.p0031	(–)
M15083	Ua	2048	2005	USA	B:ND:P1.12-1,16-8	2.27	2.p0078	(–)
M15085	41/44	5839	2006	USA	B:ND:P1.7-2,4	1.4	1.p0004	(–)
M15564	32	32	2006	USA	B:ND:P1.7,16	1.1	1.p0001	+
M16683	41/44	437	2007	USA	B:ND:5-1,2-2	2.4	2.p0019	(–)
M1820	60	60	1995	USA	B:NT:P1.5,2	1.9-3	1.p0013	(–)
M2441	269	96	1996	USA	B:NT:P1.12	3.4	3.p0031	(–)
M2552	103	103	1996	USA	B:NT:P1.18-1,3	2.10	2.p0025	(–)
M2934	32	32	1996	USA	B:15:P1.7,16	1.1	1.p0001	+
M3279	41/44	136	1997	USA	B:7:P1.17,16-3	2.9	2.p0024	(–)
M4105	41/44	154	1996	USA	B:4,7:P1.7,4	1.4	1.p0004	(–)
M4215	32	32	1996	USA	B:15:P1.7,16	1.1	1.p0001	+
M4287	41/44	44	1996	USA	B:NT:P1.7,1	2.4	2.p0019	(–)
M4717	41/44	New	1998	USA	B:14:P1.4	1.10	1.p0014	(–)
M5149	23	2421	1998	USA	B:2c:P1.5,2	2.10	2.p0025	(–)
M5258	37	916	1998	USA	B:NT:1.5,2	2.9	2.p0024	(–)
M6094	41/44	40	1999	USA	B:4,7:P1.7,13	1.10	1.p0014	(–)
M6208	103	2006	1999	USA	B:NT:P1.5-1,10-4	2.10	2.p0025	(–)
M986	11	11	1963	USA	B:2a:P1.5,2	2.7	2.p0022	+
MC58	32	74	1985	U.K.	B:15:P1.7,16b	1.1	1.p0001	+
NGP165	11	11	1974	N	B:NT:P1.2	3.2	3.p0029	+
NM008	41/44	41	1995	U.K.	B:4:P1.4	1.4	1.p0004	(–)
NM066	32	74	1997	U.K.	B:15:P1.7,16	1.1	1.p0001	+
NM092	41/44	41	1997	U.K.	B:4:P1.4	1.4	1.p0004	(–)
SWZ107	35	35	1986	CH	B:4:P1.2	2.1	2.p0016	(–)
M01-0240013	269	275	2001	U.K.	B:NT:P1.22,9	2.4	2.p0019	(–)
M01-0240149	41/44	41	2001	U.K.	B:4:P1.7,4	1.4	1.p0004	(–)

Table S1. Cont.

	Strain characterization						
	CC	ST	Year	Country	Serotype/sero-subtype	fHbp variant	*nadA* gene
M00-0243291	11	11	2000	U.K.	B:2a:P1.5,10	1.9 1.p0010	(–)
M01-0240345	60	60	2001	U.K.	B:NT:P1.21,16	1.9-3 1.p0013	(–)
M01-0240355	213	213	2001	U.K.	B:1:P1.22,14	3.4 3.p0031	+

Strain names in bold were tested in SBA with pooled sera from infant and adults. Strain names in regular type were tested with pooled sera from adults only. Ua, unassigned. Country abbreviations: AUS, Australia; C, Cuba; CAN, Canada; CH, Switzerland, D, Germany; F, France; I, Italy; N, Norway; NL, Netherlands; RUS, Russia; U.K., United Kingdom; USA, United States; CC, clonal complex; ST, sequence type.

Table S2. Comparison of different monoclonal and polyclonal antibodies for detection of different subvariants of fHbp by MATS

Strain	741 subvariant	Immunized adults (rec MenB proteins only ×3): SBA GMT post 3	Detection of fHbp in MATS (% Bmax)			
			Rabbit polyclonal	mAb JAR1	mAb JAR5	mAb 502
H44/76	1.1	73	100	100	100	100
M1390	1.14	37	15	0	39	0
MC58	1.1	35	100	100	100	100
M3812	1.12	35	12	5	19	0
NZ98/ 254	1.14	13	13	0	33	0
M6190	1.6	5	4	0	0	0

Rabbit polyclonal antiserum and three different fHbp-specific monoclonal antibodies were compared for their ability to detect different subvariants of fHbp in MATS relative to SBA on serum from human vaccinees. The rabbit polyclonal and the monoclonals JAR1 and 502 were bactericidal whereas JAR5 was not (1). MATS results were analyzed by an earlier version of the method in which the sample OD was expressed as a proportion of the OD of the H44/76 reference strain by a linear fit instead of the five-parameter logistic curve. Adult human subjects (n = 14) were immunized three times with recombinant MenB proteins only (without OMV) and were tested in SBA with human complement against the MenB strains listed. Although the pattern of reactivity of JAR5 was close to that of the polyclonal antiserum, the individual monoclonal antibodies failed to detect one or more subvariants of fHbp that were detected by the polyclonal rabbit serum and were found on strains that were killed in the SBA.

1. Welsch JA, et al. (2004) Protective activity of monoclonal antibodies to genome-derived neisserial antigen 1870, a *Neisseria meningitidis* candidate vaccine. *J Immunol* 172:5606–5615.

Table S3. Characterization of the MATS assay

	fHbp	NHBA	NadA
Acceptance criteria for regression of the reference curve, regression of the unknown curve, and parallelism analysis	$P > 0.01$	$P > 0.0001$	$P > 0.05$
Lower limit of quantitation (LLOQ)	1.67%	20.2%	0.17%
Intermediate precision (% CV)	20%	27%	34%
Accuracy (relative bias)	−1.8%	−1.7%	−1.4%

Eighteen strains, covering the whole range of MATS relative potency for the three antigens, were tested 6–10 times in different days and by different operators. Analysis of the statistical reports produced by StatLIA for single experiments led to the definition of acceptance criteria for the three main data reduction steps: regression of the five-parameters logistic curve (5-PL) to reference strain optical density, regression of the 5-PL to unknown strain optical density, and test for parallelism of the two 5-PL curves. StatLIA produces for each step a single P value, based on a collection of historical experiments and on Monte Carlo simulations. In the first row are reported the limiting values for these P values: if an assay produces a P value lower than the values reported, it is rejected. Acceptable results were then analyzed to determine the coefficient of variation (% CV) for each strain/antigen. Values of % CV were found to be relatively stable for high, medium, and low values of the relative potency, but below a certain value % CV was found to increase significantly to values >50%. These values were defined as the lower limit of quantitation (LLOQ) for the assay and are reported in the second row. The intermediate precision of the assay, for each antigen, was defined as the highest % CV measured for the strain with mean relative potency above the LLOQ and is reported in the third row. Accuracy of the assay was determined as the relative bias comparing the relative potency determined for the reference strains compared with themselves. The relative bias was calculated as $100 \cdot \left(\frac{\text{Relative Potency}}{100} - 1\right)\%$, and results are reported in the bottom row.