Table 2. Concordance between the electrochemiluminescence assay and the WHO reference ELISA.

Serotype	N1[†]	Concordance slope		Average % difference		Fold difference at 0.35 μg/ml[‡]	Agreement coefficient		
		Slope	95% CI	% difference	95% CI		Correlation	Accuracy	Concordance
1	91	1.05	1.00–1.11	10.3	4.8–16.0	1.09	0.97	0.99	0.96
3	73	1.17	0.95–1.43	3.5	-11.5–21.0	1.00	0.75	0.99	0.75
4	75	1.11	1.04–1.18	15.4	9.4–21.8	1.11	0.97	0.98	0.95
5	102	1.07	0.93–1.22	52.1	40.6–64.6	1.49	0.83	0.84	0.70
6A	89	1.29	1.13–1.46	-10.3	-20.2–0.8	0.74	0.86	0.97	0.83
6B	93	1.05	1.00–1.10	-4.3	-8.6–0.2	0.91	0.97	1.00	0.97
7F	107	0.99	0.95–1.03	22.4	18.4–26.6	1.23	0.98	0.98	0.96
9V	88	1.33	1.22–1.46	27.8	16.8–39.7	1.20	0.92	0.93	0.86
14	109	0.97	0.94–1.01	20.9	16.0–26.0	1.26	0.98	0.99	0.97
18C	77	1.11	1.04–1.18	28.6	22.5–35.0	1.26	0.96	0.94	0.90
19A	94	1.28	1.14–1.43	-12.7	-21.8 to -2.5	0.77	0.87	0.97	0.85
19F	109	1.22	1.15–1.28	-21.4	-27.2 to -15.1	0.63	0.96	0.96	0.93
22F	116	1.05	0.98–1.13	1.5	-5.2–8.7	0.94	0.94	1.00	0.93
23F	72	1.08	1.00–1.17	-7.8	-13.9 to -1.2	0.89	0.95	0.99	0.95
33F	116	1.01	0.97–1.06	-8.0	-11.4 to -4.4	0.91	0.97	1.00	0.97
Overall	1411	1.08	1.05–1.10	6.0	3.7–8.2	1.01	0.93	1.00	0.93

[†]N1 is the number of test samples with quantifiable concentrations in both assays, used to estimate the concordance slope and the average % difference.
[‡]To calculate the fold difference at the serostatus threshold of 0.35 μg/ml, 0.35 μg/ml was used as the concentration of the WHO ELISA.
ECL: Electrochemiluminescence.

concentration between the Pn ECL and the WHO reference ELISA was between -22 and 29%. The fold difference between the assays at 0.35 μg/ml ranged from 0.63 (serotype 19F) to 1.49 (serotype 5), with an overall fold difference of 1.01 when taken over all 15 serotypes.

For the set of pediatric samples, the serotype-specific Pn ECL threshold values equivalent to the WHO reference ELISA value of 0.35 μg/ml obtained using the concordance method ranged from 0.22 to 0.52 μg/ml, with an aggregate threshold of 0.35 μg/ml across all 15 serotypes, whereas the RCDF method produced threshold values ranging from 0.24 to 0.56 μg/ml (Supplementary Figure 5), with an aggregate value of 0.38 μg/ml (Figure 2). The RCDF curves display the proportion of subjects tested whose serotype concentration exceeded a specified concentration.

The rate of agreement in serostatus assignment was assessed on test samples using the assay-specific median concentrations for each test sample. Using the serostatus threshold of 0.35 μg/ml for the Pn ECL and the WHO reference ELISA assays, the agreement rates in serostatus assignment were greater than 80% for all serotypes in the pediatric sample panel. Using either the concordance threshold value or the RCDF threshold value for individual serotypes resulted in only a slight improvement in serostatus agreement rates as compared with the 0.35 μg/ml threshold. Using a serostatus threshold of 0.35 μg/ml for both assays, the 2 × 2 cross-classification tables for the set of pediatric samples are provided by serotype in Table 3. Across the 15 serotypes, the agreement rate in serostatus between the two assays ranged from 80.2 to 96.6% and Cohen's κ-coefficient ranged from 0.474 to 0.908, with aggregate values of 89.4% and 0.782, respectively (Table 3). The overall McNemar's exact p-value was 0.046.

For the subset of pediatric samples having WHO reference ELISA concentrations close to 0.35 μg/ml, the serostatus agreement rate using the 0.35 μg/ml threshold for the Pn ECL ranged from 59.5% to 87.9% across the 15 serotypes. For this subset of samples, using either the concordance threshold value or the RCDF threshold value for individual serotypes as compared with the 0.35 μg/ml threshold resulted in improvement in serostatus agreement rates of ≥8 percentage points in six of the 15 serotypes (data not shown).

For the panel of 12 adult samples (Goldblatt panel), ≥75% of samples had concentrations within ±40% of the published values [7] for eight of the 13 serotypes with the WHO reference ELISA; there are no published results for serotypes 22F and 33F in the panel of adult sera. For the other five serotypes, 42–58% of samples had concentrations within ±40% of the published values. For the Pn ECL assay, ≥75% of samples had concentrations within ±40% of the published values for six of the 13 serotypes; while 42–67% of samples were within ±40% of