Creation, Characterization, and Assignment of Opsonic Values for a New Pneumococcal OPA Calibration Serum Panel (Ewha QC Sera Panel A) For 13 Serotypes

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Abstract

Background. Opsonophagocytic assays (OPAs) are useful for assessing the immunogenicity of pneumococcal vaccines, especially in the elderly. To reduce the variability in OPA results from different laboratories, sera with known OPA values are needed for assay calibration. Although a serum panel was created by the US FDA, those sera are in limited quantities and are not available. Therefore, a new panel (Ewha QC Sera Panel A) was created and an international collaborative study was conducted to determine consensus values for the 13 serotypes in PCV13.

Methods. Sera were collected from 20 healthy adults after PPV23 vaccination, lyophilized, and aliquoted into at least 150 vials per serum. Four laboratories tested the sera five times, with reference serum pool 007sp included in each run. For each result, an unadjusted opsonic index (OI) and a normalized OI (based on 007sp performance) were calculated. The consensus values of both the unadjusted OIs and the normalized OIs were estimated using an ANOVA model.

Results. The results for one laboratory differed significantly from those of the other laboratories and were therefore excluded from consensus value determination. Using data from the three remaining laboratories, consensus OIs (both unadjusted and normalized) were determined for each serum sample for 13 serotypes.

Conclusion. Ewha QC Serum Panel A will be useful for calibrating pneumococcal OPAs. The sera can be obtained by contacting Kyung-Hyo Kim (kaykim@ewha.ac.kr) or Si Hyung Yoo (yoosh1130@korea.kr) in Korean MFDS.

Introduction

Conjugate vaccines targeting the pneumococcal capsule have been quite effective in reducing the burden of disease caused by serotypes covered by the current vaccines. However, as the distribution of pneumococcal serotypes changes, new formulations of conjugate vaccines must be developed. Due to the costs and ethical concerns associated with true vaccine efficacy studies, prospective vaccines may be licensed based on immunogenicity. Opsonophagocytic killing assays (OPAs) are often used to assess the relative immunogenicity of a prospective vaccine, especially in adult populations.

Over the past 15 years, significant improvements have been made to pneumococcal OPAs, resulting in reliable assays that are practical for use in support of vaccine trials. However, a 2011 study [1] found that although OPA results from different laboratories correlated reasonably well, the absolute agreement was quite low. In a follow-up study [2], it was shown that normalization with a reference sera (007sp) significantly reduced the inter-laboratory variability.

The follow-up study [2] also yielded a serum panel that could be used to calibrate pneumococcal OPAs. However, the availability of these sera was significantly limited due to low volumes. Thus, an additional international collaborative study involving 4 laboratories familiar with OPAs was devised to: 1) determine consensus values for an OPA calibration panel that could be readily distributed; and 2) determine if normalization of results with 007sp had any impact on the inter-laboratory agreement of OPA results.

Methods

Participating laboratories. For laboratories participated in this study: Ewha Womans University (Seoul, Republic of Korea), Murdoch Childrens Research Institute (Victoria, Australia), SK Chemicals (Gyeonggi-do, Republic of Korea), and the University of Alabama at Birmingham (Birmingham, AL, USA). Labs are listed alphabetically and this order does not correspond to lab designations A through D.

Sera. Pneumococcal reference serum pool 007sp has been described previously (1). For the 20 Ewha QC sera, donors were vaccinated with PPV23 and blood was collected on 2 or 3 occasions, with the first collection ~1 month (15 donors) or ~4 years (5 donors) after vaccination.

Study design. Each laboratory tested the twenty calibration sera in five separate runs, with 007sp included once in each run.

OPAs. All participating laboratories used a multiplexed OPA (MOPA) format. Each laboratory converted raw colony counts to opsonic indexes (OIs) using Opsotiter template, with an OI defined as the estimated dilution of serum that kills 50% of the target bacteria. Results that failed to meet a laboratory's criteria were indicated as "IR". Samples with undetectable OIs (ie, OIs <8) were assigned a value of "4" and were not normalized.

Statistical analyses. Normalized OIs for the calibration sera were obtained using the formula:

$$Normalized\ OI = Unadjusted\ OI\ X\ \frac{007sp\ assigned\ OI}{007sp\ OI\ from\ run}$$

To estimate the non-normalized and normalized consensus OIs for the calibration sera, the log transformed OIs (unadjusted or normalized) were fit by serotype and sample using a mixed effects ANOVA model consisting of the random terms Lab and Run(Lab). Consensus OIs and corresponding 95% confidence intervals (CIs) for the calibration sera were obtained by back-transforming the obtained model intercept and its corresponding 95% CI.

Results

Figure 1. 007sp Results of Individual Laboratories. For the 13 target serotypes (x axis), the laboratory-specific geometric mean 007sp OI (GMOI, y axis) is shown for each participating laboratory (see legend). The assigned 007sp OI (solid horizontal lines, [1]), as well as 3-fold deviations (dashed vertical lines) from the assigned value, for each serotype are also shown.

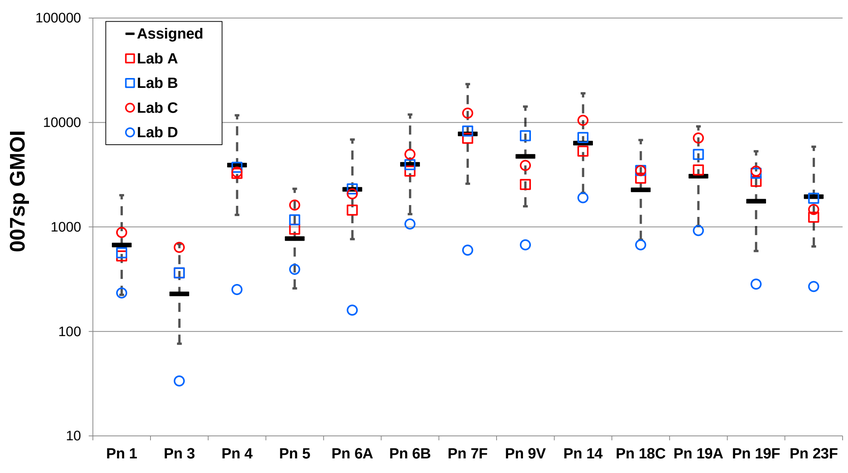


Table 1. Model-based assessment of the effect of normalization (with Lab D data). The overall reduction in variability due to normalization is shown for each serotype. Estimates of CVs of the unadjusted and normalized results from the ANOVA are also shown.

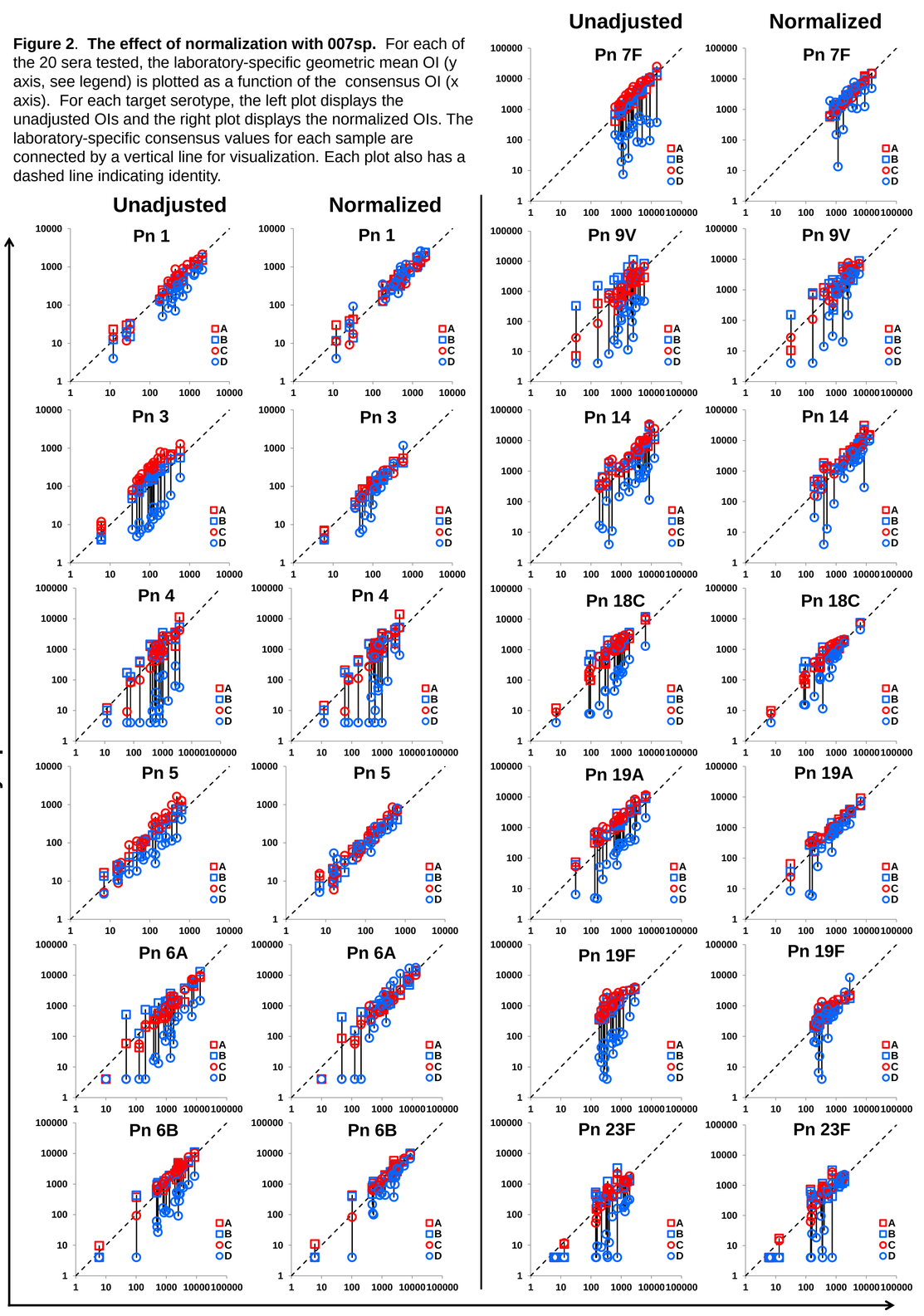
	%CV Unadjusted	%CV Normalized	% Variability Reduction
Pn 1	74%	66%	3%
Pn 3	233%	79%	50%
Pn 4	1048%	180%	51%
Pn 5	91%	64%	22%
Pn 6A	316%	130%	33%
Pn 6B	218%	103%	37%
Pn 7F	444%	109%	48%
Pn 9V	514%	176%	36%
Pn 14	348%	162%	31%
Pn 18C	308%	124%	37%
Pn 19A	259%	98%	44%
Pn 19F	451%	123%	45%
Pn 23F	356%	155%	33%

Table 2. Normalized Calibration Sera Consensus OIs (without Lab D). For each serum in the panel, the consensus OI and the corresponding 95% confidence internal are shown for the indicated serotypes. Results in red text indicate at least one laboratory reported an irregular result for at least one run.

		Pn 1	Pn 3	Pn 4	Pn 5	Pn 6A	Pn 6B	Pn 7F	Pn 9V	Pn 14	Pn 18C	Pn 19A	Pn 19A	Pn 23F
QC-01	Consensus OI	566	463	108	213	2883	2300	1827	1017	5029	834	1170	555	1326
	(95% CI)	(217, 1475)	(329, 652)	(80, 145)	(133, 342)	(1663, 4999)	(1052, 5026)	(1227, 2719)	(618, 1674)	(3250, 7783)	(533, 1304)	(624, 2194)	(348, 884)	(931, 1890)
QC-02	Consensus OI	236	52	859	68	57	5	2458	388	3855	330	3720	879	4
	(95% CI)	(122, 455)	(34, 80)	(82, 9041)	(34, 137)	(0, 14744)	(1, 30)	(1754, 3445)	(23, 6430)	(2176, 6830)	(189, 575)	(2911, 4754)	(522, 1481)	(NA)
QC-03	Consensus OI	22	48	9	6	933	1064	844	1136	471	1126	1428	208	1154
	(95% CI)	(5, 93)	(28, 82)	(2, 44)	(1, 50)	(532, 1634)	(670, 1689)	(418, 1704)	(442, 2916)	(275, 807)	(454, 2792)	(642, 3174)	(156, 279)	(557, 2390)
QC-04	Consensus OI	351	4	1276	584	4	246	960	42	1037	388	399	312	4
	(95% CI)	(188, 653)	(1, 13)	(1015, 1604)	(262, 1300)	(NA)	(27, 2265)	(588, 1566)	(2, 869)	(539, 1996)	(190, 791)	(206, 773)	(190, 511)	(NA)
QC-05	Consensus OI	16	3	72	14	1451	1242	604	850	316	141	382	226	412
	(95% CI)	(4, 63)	(1, 12)	(1, 4776)	(4, 42)	(822, 2561)	(877, 1758)	(380, 959)	(414, 1747)	(128, 777)	(43, 456)	(204, 717)	(142, 359)	(224, 759)
QC-06	Consensus OI	1204	105	1511	121	1802	3177	2259	2733	3679	908	844	420	1285
	(95% CI)	(751, 1930)	(53, 211)	(1122, 2033)	(58, 251)	(1017, 3192)	(1865, 5412)	(1195, 4268)	(1306, 5719)	(1846, 7330)	(647, 1275)	(577, 1236)	(220, 801)	(886, 1864)
QC-07	Consensus OI	2006	181	3053	420	1379	2383	6686	7029	8938	163	957	911	951
	(95% CI)	(1289, 3122)	(140, 233)	(1991, 4679)	(262, 673)	(987, 1929)	(1746, 3254)	(5486, 8150)	(3981, 12411)	(6146, 12998)	(20, 1346)	(791, 1158)	(643, 1291)	(621, 1458)
QC-08	Consensus OI	308	117	1438	13	12312	9392	1201	4541	1352	1057	1128	636	2643
	(95% CI)	(198, 479)	(75, 182)	(1119, 1847)	(5, 37)	(7428, 20408)	(7122, 12384)	(780, 1850)	(3220, 6404)	(718, 2547)	(493, 2266)	(672, 1893)	(316, 1282)	(1701, 4106)
QC-09	Consensus OI	21	179	2269	52	677	720	8644	1834	1325	7	392	999	688
	(95% CI)	(3, 153)	(112, 287)	(712, 7232)	(19, 141)	(452, 1015)	(459, 1129)	(5031, 14854)	(773, 4354)	(955, 1837)	(0, 3451)	(208, 737)	(439, 2274)	(363, 1303)
QC-10	Consensus OI	1180	112	841	30	902	3079	3969	934	1486	376	287	215	1489
	(95% CI)	(493, 2823)	(68, 185)	(524, 1351)	(8, 111)	(449, 1812)	(2011, 4715)	(2914, 5405)	(600, 1455)	(868, 2543)	(143, 991)	(223, 369)	(153, 301)	(806, 2752)
QC-11	Consensus OI	1362	32	652	56	503	619	874	1512	3195	1082	6355	447	644
	(95% CI)	(827, 2242)	(20, 54)	(370, 1151)	(18, 177)	(216, 1173)	(327, 1169)	(552, 1382)	(718, 3187)	(1220, 8369)	(597, 1959)	(2918, 13839)	(236, 845)	(266, 1557)
QC-12	Consensus OI	441	108	7105	271	374	1803	2254	2534	13646	1372	641	808	334
	(95% CI)	(294, 663)	(77, 150)	(1694, 29798)	(176, 416)	(114, 1221)	(1223, 2659)	(1242, 4091)	(1648, 3897)	(8759, 21257)	(629, 2989)	(410, 1002)	(344, 1901)	(141, 788)
QC-13	Consensus OI	907	136	1326	580	2339	2407	9645	1545	8030	1387	2358	1394	310
	(95% CI)	(498, 1655)	(93, 200)	(755, 2329)	(241, 1397)	(1299, 4212)	(1745, 3320)	(5145, 18081)	(628, 3799)	(3593, 17949)	(847, 2272)	(1358, 4094)	(853, 2278)	(11, 8749)
QC-14	Consensus OI	372	318	589	11	7290	3981	3242	3655	5204	7225	2056	1730	1538
	(95% CI)	(227, 607)	(131, 770)	(390, 890)	(2, 74)	(4217, 12602)	(3135, 5055)	(1336, 7868)	(928, 14397)	(3197, 8471)	(4846, 10773)	(1050, 4025)	(1258, 2380)	(954, 2478)
QC-15	Consensus OI	565	201	3729	184	790	922	1777	4817	2509	967	725	496	258
	(95% CI)	(309, 1034)	(97, 419)	(2643, 5260)	(109, 313)	(270, 2307)	(383, 2221)	(990, 3189)	(2506, 9259)	(929, 6780)	(511, 1829)	(377, 1394)	(291, 847)	(14, 4850)
QC-16	Consensus OI	495	238	2665	156	2035	3730	4602	6068	24471	1006	3174	1011	697
	(95% CI)	(333, 737)	(155, 366)	(1859, 3819)	(90, 273)	(1274, 3251)	(2691, 5170)	(2327, 9100)	(3649, 10091)	(14373, 41662)	(503, 2014)	(2380, 4233)	(535, 1908)	(484, 1004)
QC-17	Consensus OI	756	61	716	74	83	841	1157	325	1803	295	204	291	223
	(95% CI)	(468, 1220)	(30, 125)	(319, 1605)	(47, 116)	(24, 286)	(584, 1212)	(684, 1956)	(139, 764)	(1213, 2680)	(164, 528)	(77, 539)	(163, 519)	(108, 459)
QC-18	Consensus OI	142	103	700	15	6349	5964	14734	3452	8147	1970	819	575	1805
	(95% CI)	(83, 243)	(56, 188)	(478, 1025)	(8, 31)	(3469, 11620)	(3676, 9677)	(9111, 23829)	(969, 12303)	(3168, 20949)	(1412, 2749)	(655, 1023)	(388, 851)	(904, 3603)
QC-19	Consensus OI	400	121	274	248	443	4673	1428	922	282	694	38	288	480
	(95% CI)	(238, 671)	(82, 179)	(45, 1693)	(203, 305)	(98, 2007)	(2829, 7718)	(808, 2524)	(179, 4748)	(73, 1086)	(539, 894)	(11, 136)	(156, 530)	(255, 904)
QC-20	Consensus OI	421	68	1291	149	1446	3417	4508	3510	6937	631	817	1997	10
	(95% CI)	(220, 806)	(39, 117)	(802, 2076)	(74, 301)	(771, 2713)	(1914, 6100)	(2687, 7565)	(1713, 7191)	(4081, 11792)	(418, 952)	(434, 1537)	(1436, 2777)	(1, 79)

Pn, pneumococcal serotype; OI, Opsonic Index; CI, Confidence Interval; NA, Not Applicable (all reported values were undetectable and/or irregular)

Figure 2. The effect of normalization with 007sp. For each of the 20 sera tested, the laboratory-specific geometric mean OI (y axis, see legend) is plotted as a function of the consensus OI (x axis). For each target serotype, the left plot displays the unadjusted OIs and the right plot displays the normalized OIs. The laboratory-specific consensus values for each sample are connected by a vertical line for visualization. Each plot also has a dashed line indicating identity.



Conclusions

1. The Ewha QC Sera Panel A sera will be an important tool for setting up new OPAs and for calibrating existing OPAs.
2. As with a previous study [2], normalization with 007sp decreased the interlaboratory variability between the 4 laboratories. However, the absolute variabilty remained high for some serotypes, mostly driven by the Lab D data. For many serotypes, the 007sp results from Lab D were quite lower than the assigned values, indicating that perhaps a minimum level of agreement with the assigned 007sp values may be needed to full reap the benefits of normalization.

References

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