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Creation, characterization, and assignment of opsonic values for a new pneumococcal OPA calibration serum panel (Ewha QC sera panel A) for 13 serotypes

Robert L. Burton, MS[a], Han Wool Kim, MD, PhD[b,c], Soyoung Lee, MD, PhD[b,c], Hun Kim, PhD[d], Jee-Hyun Seok, MS[d], Sang Heon Lee, MS[e], Anne Balloch, MS[f], Paul Licciardi, BS[f], Rachel Marimla, BS[f], Sejong Bae, PhD[a], Moon H. Nahm, MD[a], Kyung-Hyo Kim, MD, PhD[b,c,*]

Abstract

Pneumococcal conjugate vaccines (PCVs) have been very effective in reducing the disease burden caused by *Streptococcus pneumoniae* serotypes covered by the current vaccine formulations. However, the incidence of disease caused by serotypes not covered by the vaccine is increasing. Consequently, there are active efforts to develop new PCVs with additional serotypes in order to provide protection against the emergent serotypes. Due to costs and ethical issues associated with performing true vaccine efficacy studies, new PCVs are being licensed based on their immunogenicity, which may be assessed with 2 in vitro assays: enzyme-linked immunosorbent assay (ELISA) for quantitating antibody level and opsonophagocytic assay (OPA) for assessing protective function. While a standardized ELISA has been developed, OPA results from different laboratories can be quite disparate, even among laboratories utilizing the same platform. In order to harmonize OPA data, a recent international collaboration assigned opsonic indices to the US Food and Drug Administration (US FDA) reference serum, 007sp, as well as a panel of US FDA calibration sera. However, due to a low number of aliquots, the availability of these calibration sera is extremely limited. Because calibration sera are critical to establish the performance characteristics of an OPA, a second calibration serum panel was created, comprised of 20 sera collected from adults immunized with the 23-valent polysaccharide vaccine, with 150 to 500 aliquots prepared for each serum. In order to establish consensus OPA values of the 20 sera for the 13 serotypes in 13-valent PCV, the sera were tested by 4 laboratories in an international collaborative OPA study. The 007sp results of 1 laboratory deviated significantly from those obtained by the other laboratories, as well as from previously assigned values. Due to these discrepancies, the consensus values for the calibration sera were determined based on the data from the remaining laboratories. Thus, we were able to create a panel of sera with consensus opsonic values that could be used by outside laboratories to calibrate pneumococcal OPAs. Our results also confirmed findings of a previous study that normalization of OPA results significantly reduces interlaboratory variation, with normalization based on 007sp reducing variation by 43% to 74%, depending on serotype.

Abbreviations: CI = confidence interval, CV = coefficient of variation, ELISA = enzyme-linked immunosorbent assay, GMOI = geometric mean opsonic index, IPD = invasive pneumococcal disease, OI = opsonic index, OPA = opsonophagocytic assay, PCV = pneumococcal conjugate vaccine, PPSV23 = 23-valent pneumococcal polysaccharide vaccine, PS = polysaccharide, US FDA = United States Food and Drug Administration.

Keywords: 007sp, opsonophagocytic assay, pneumococcus, quality control sera, standardization, vaccines

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[a] Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, [b] Department of Pediatrics, Ewha Womans University College of Medicine, [c] Center for Vaccine Evaluation and Study, Medical Research Institute, Ewha Womans University College of Medicine, Seoul, [d] Bio R&D, Global BD, Life Science R&D Center, SK Chemicals, Seongnam-Si, Gyeonggi-do, [e] Vaccine R&D Center, Life Sciences R&D, Life Sciences Company, LG Chem Ltd, Daejeon, Republic of Korea, [f] Pneumococcal Research Group, Murdoch Children's Research Institute, Royal Children's Hospital, Victoria, Australia.

[*] Correspondence: Kyung-Hyo Kim, Department of Pediatrics, Ewha Womans University College of Medicine, 1071, Anyangcheon-ro, Yangcheon-gu, Seoul, Korea 07985 (e-mail: kaykim@ewha.ac.kr).

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1. Introduction

Streptococcus pneumoniae is a gram positive, commensal bacterial species capable of causing serious diseases in humans, especially those younger than 2 and older than 65 years of age. Pneumococcal conjugate vaccines (PCVs), prepared by conjugating capsular polysaccharide (PS) to carrier proteins, have been quite effective in reducing the incidence of invasive pneumococcal diseases (IPDs) caused by the vaccine serotypes in both children and old adults.[1] With the use of PCVs, however, the incidence of IPDs caused by nonvaccine serotypes has significantly increased,[1,2] spurring the development of new PCVs with additional serotypes.

Due to the overall low incidence of IPD, efficacy trials for new PCVs would require impractically large clinical trials, and in many cases would not be ethically possible due to current PCV usage. Thus, efficacy of the newly formulated PCVs is estimated by quantitating antibodies against capsular PS using 2 in vitro immunoassays: enzyme-linked immunosorbent assay (ELISA) and opsonophagocytic assay (OPA). ELISA has been used extensively to study immune responses to PCVs, especially among pediatric populations.[3] However, old adults tend to have high antibody concentrations prior to vaccination,[4] and ELISA results failed to predict clinical protection for some serotypes.[5,6] Since the OPA mimics the in vivo mechanism of protection against pneumococcal infections, OPA results are better surrogates of immune protection. Therefore, OPAs are needed for vaccine evaluations.

Early OPAs were labor intensive and therefore not suitable for evaluating large numbers of sera from clinical trials. However, many laboratories, both academic and industrial, have now developed high throughput OPAs.[7–9] Furthermore, although OPA results from different laboratories vary significantly and are therefore difficult to compare,[10] a recent study suggested that normalization of OPA results from different laboratories with a reference serum ("007sp") significantly reduced the interlaboratory variation.[11] While the reference serum, 007sp, is readily available, the calibration sera in the panel characterized in that study are limited in quantity and are not generally available. The goals of the current study were to produce a new set of calibration sera with consensus values for a general use and to confirm the benefit of normalization previously reported.

2. Methods

2.1. Laboratories

The laboratories participating in this study are listed alphabetically in Table 1. This order does not reflect the anonymized laboratory letter designations used throughout this report.

2.2. Sera

The preparation of pneumococcal reference serum 007sp has been described previously.[12]

Table 1

Participating laboratories and opsonophagocytic assay formats.

Institution	Location	OPA format and reference
Ewha Womans University	Seoul, Korea	MOPA[8]
Murdoch Children's Research Institute	Parkville, Victoria, Australia	MOPA[8]
SK Chemical	Seongnam-si, Korea	MOPA[8]
University of Alabama at Birmingham	Birmingham, AL	MOPA[8]

MOPA = opsonophagocytic assay with a multiplexed format, OPA = opsonophagocytic assay.

To create the Korea OPA Calibration Serum Panel A, 63 individuals were evaluated at the Ewha Center for Vaccine Evaluation and Study, Ewha Womans University College of Medicine after written informed consent. Twenty healthy male and nonpregnant female volunteers between 20 and 50 years of age met the eligibility requirements for this study. Eligibility was determined by a physical assessment and a questionnaire concerning medical history and risk factors associated with exposure to, or clinical evidence of, a relevant transfusion-transmitted infection. Participants were negative for hepatitis B, hepatitis C, and HIV. The study was approved by the Ewha Womans University Mokdong Hospital institutional review board (EUMC 2015-01-062-001). Fifteen volunteers were vaccinated once with 23-valent pneumococcal polysaccharide vaccine (PPSV23) (Prodiax23, Merck & Co. Inc., Whitehouse Station, NJ) by intramuscular injection, and donated a unit of blood 14 to 27 days following immunization and a second unit of blood 8 to 12 weeks after the first donation (see Table 2). Five volunteers who were vaccinated previously (46–50 months prior) with PPSV23 (Prodiax23) donated a unit of blood, a second unit of blood 8 to 12 weeks later, and in some instances a third unit of blood 8 to 12 weeks after the second donation (Table 2). Blood was allowed to clot and the serum was collected and stored at −80°C at the Ewha Center for Vaccine Evaluation and Study. For each donor, the sera from the 2 blood donations were thawed, pooled, and 1-mL aliquots were prepared (153–534 vials were prepared for each of the 20 sera). The aliquots were lyophilized by LG Life Sciences R&D (Daejeon, Republic of Korea) and are stored at ≤ -70°C.

2.3. Study design

Each participating laboratory tested the 20 calibration sera in 3 to 5 separate runs with 007sp included at least once in each run. Each participating laboratory used its own batches/lots of reagents.

Table 2

Donor information.

Sample ID	Donor age, y	Interval postvaccination (donation 1)
PnQC-01	29	46 mo
PnQC-02	46	47 mo
PnQC-03	46	47 mo
PnQC-04	30	48 mo
PnQC-05	30	50 mo
PnQC-06	45	14 d
PnQC-07	20	21 d
PnQC-08	33	14 d
PnQC-09	39	15 d
PnQC-10	43	18 d
PnQC-11	44	19 d
PnQC-12	31	20 d
PnQC-13	25	21 d
PnQC-14	47	27 d
PnQC-15	20	21 d
PnQC-16	20	14 d
PnQC-17	42	21 d
PnQC-18	41	16 d
PnQC-19	49	20 d
PnQC-20	47	26 d

For each calibration serum, the age and the postvaccination interval of the donor are shown in the table.

2.4. OPAs

All participating laboratories utilized the multiplexed OPA format.[8] Briefly, target bacteria were thawed, washed with opsonization buffer B (Hanks' balanced salt solution with 0.1% gelatin and 5% fetal bovine serum), and diluted (to ~5×10^4 CFU/mL). Ten microliters of diluted bacteria were added to 20 μL of serially diluted sera and assay plates were incubated for 30 min at room temperature with shaking. Baby rabbit complement (final 12.5%) and dimethylformamide-differentiated HL60 cells (4×10^5 cells/well) were added for a total assay volume of 80 μL. Plates were incubated for 45 min at 37°C/5% CO_2 with shaking. After incubation, plates were placed on ice for at least 10 to 20 min. A 10-μL aliquot of the final reaction mixture was spotted onto agar plates (Todd–Hewitt broth with 0.5% yeast extract and 1.5% agar). An equal volume of overlay agar (Todd–Hewitt broth with 0.5% yeast extract and 0.75% agar) containing 25 mg/L and the selective antibiotic was added, and the plates were incubated overnight at 37°C/5% CO_2. A detailed procedure can be found at https://www.vaccine.uab.edu/UAB-MOPA.pdf (Lab D made minor modifications to the protocol including shaking assay plates at 220 rpm, rather than the speed specified in the protocol, 700 rpm).

After overnight incubation, the number of surviving colonies was determined. Each laboratory converted raw colony counts to opsonic indexes (OIs) using the same MS Excel-based template ("Opsotiter").

Reference serum 007sp was included once in each run.

2.5. Statistical analyses

Statistical analyses were performed as described previously.[11] Briefly, calibration sera OIs were normalized using the following formula:

$$\left(\text{Normalized OI} = \text{Unadjusted OI} \times \frac{007\,\text{sp assigned OI}}{007\,\text{sp OI from run}}\right)$$

Consensus OIs (unadjusted and normalized) for the calibration sera were estimated for serotype and sample by fitting the log transformed OIs using a mixed-effect analysis of variance model consisting of the random terms Lab and Run(Lab). Consensus OIs and the corresponding 95% confidence intervals (CIs) were obtained by back-transforming the model intercept and its corresponding CI.

For individual serotypes (except serotypes 1 and 23F), the percent reduction in interlaboratory variability due to normalization was calculated as:

$$\%\,\text{Reduction} = \left(1 - \frac{\ddot{\sigma}_L^2 + \ddot{\sigma}_{R(L)}^2 + \ddot{\sigma}_{S\times L}^2 + \ddot{\sigma}_{S\times R(L)}^2}{\dot{\sigma}_L^2 + \dot{\sigma}_{R(L)}^2 + \dot{\sigma}_{S\times L}^2 + \dot{\sigma}_{S\times R(L)}^2}\right) \times 100\%$$

with $\dot{\sigma}_L^2$, $\dot{\sigma}_{R(L)}^2$, $\dot{\sigma}_{S\times L}^2$, and $\dot{\sigma}_{S\times R(L)}^2$ defined as the interlaboratory, run-within-laboratory, sample-by-laboratory, and sample-by-run-within-laboratory variance component estimates for the unadjusted OIs, respectively; and $\ddot{\sigma}_L^2$, $\ddot{\sigma}_{R(L)}^2$, $\ddot{\sigma}_{S\times L}^2$, and $\ddot{\sigma}_{S\times R(L)}^2$ defined as the corresponding variance components for the normalized OIs.

For serotypes 1 and 23F, the variability actually increased slightly after normalization. For these serotypes, the percent "reduction" was calculated as:

$$\%\,\text{Reduction} = \left(\frac{\dot{\sigma}_L^2 + \dot{\sigma}_{R(L)}^2 + \dot{\sigma}_{S\times L}^2 + \dot{\sigma}_{S\times R(L)}^2}{\ddot{\sigma}_L^2 + \ddot{\sigma}_{R(L)}^2 + \ddot{\sigma}_{S\times L}^2 + \ddot{\sigma}_{S\times R(L)}^2} - 1\right) \times 100\%$$

with the same term definitions as above.

3. Results

3.1. OIs obtained for 007sp

The 007sp geometric mean opsonic index (GMOI) obtained by each laboratory is shown in Table 3 and Fig. 1. Generally, the results obtained by Labs A, B, and C were comparable to each other as well as to the assigned values, with the Lab C results trending slightly higher than those of Labs A and B. However, most of the results obtained by Lab D were significantly lower than the other laboratories as well as the assigned values, with the results of multiple serotypes differing by more than 10-fold. The exceptions were serotypes 1 and 5, for which the results from Lab D were within 3-fold and 2-fold, respectively, of the assigned values.

Table 3

007sp geometric mean opsonic indices.

	Assigned	Lab A		Lab B		Lab C		Lab D	
	OI[11]	GMOI	Ratio	GMOI	Ratio	GMOI	Ratio	GMOI	Ratio
Pn 1	672	527	0.78	566	0.84	885	1.32	234	0.35
Pn 3	229	363	1.58	363	1.58	638	2.79	33	0.15
Pn 4	3912	3256	0.83	3715	0.95	3347	0.86	252	0.06
Pn 5	774	950	1.23	1169	1.51	1616	2.09	393	0.51
Pn 6A	2293	1448	0.63	2308	1.01	2072	0.90	160	0.07
Pn 6B	3976	3418	0.86	3942	0.99	4958	1.25	1065	0.27
Pn 7F	7776	7051	0.91	8267	1.06	12,258	1.58	599	0.08
Pn 9V	4733	2550	0.54	7466	1.58	3878	0.82	674	0.14
Pn 14	6349	5306	0.84	7177	1.13	10,466	1.65	1904	0.30
Pn 18C	2264	2930	1.29	3473	1.53	3463	1.53	674	0.30
Pn 19A	3059	3509	1.15	4945	1.62	7095	2.32	922	0.30
Pn 19F	1766	2728	1.54	3260	1.85	3425	1.94	283	0.16
Pn 23F	1952	1239	0.63	1881	0.96	1469	0.75	269	0.14

The GMOIs obtained by each laboratory, as well as the ratio of the GMOI to the assigned value for each laboratory, are indicated.
GMOI = geometric mean opsonic index, OI = opsonic index, Pn = pneumococcal serotype.

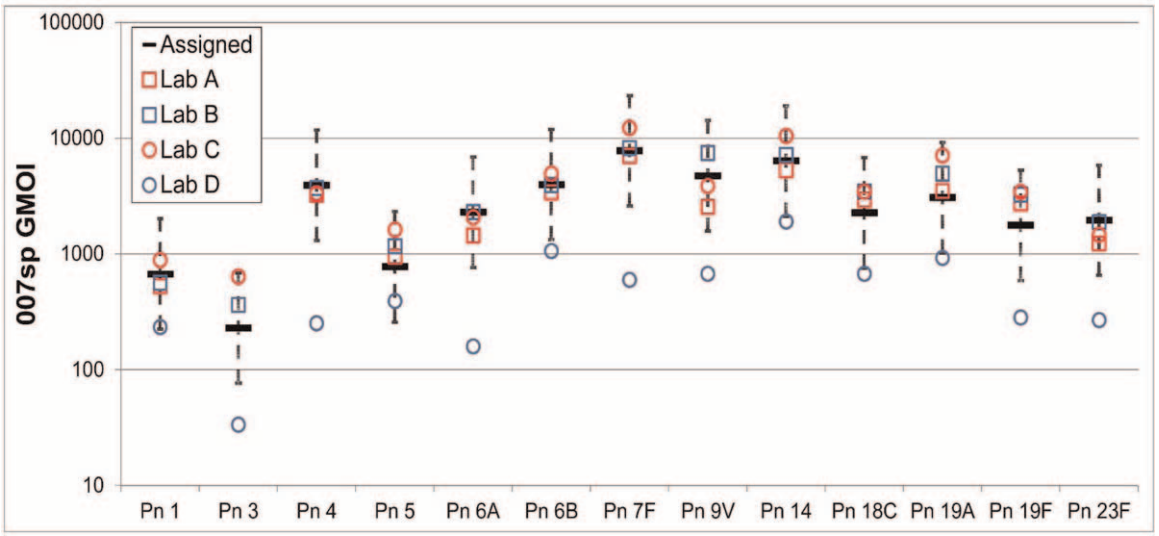

Figure 1. 007sp opsonic indices. The 007sp GMOIs obtained by each laboratory (color symbol) and the assigned OIs for 007sp (black horizontal line) are shown for each target serotype. The dashed vertical lines indicate 3-fold deviations from the assigned OI (see Section 4). GMOI = geometric mean opsonic index, OI = opsonic index, Pn = pneumococcal serotype.

3.2. Effect of standardization on calibration sera results

For Labs A, B, and C, the unadjusted OIs for the calibration sera agreed reasonably well (the GMOIs for each laboratory are shown in Supplementary Table 1, http://links.lww.com/MD/C217). The coefficients of variation (CVs) for the unadjusted values were <60% for all serotypes except 4 (81%), 6A (96%), 9V (111%), and 23F (64%) with the exclusion of the data from Lab D (Table 4). By contrast, when the data from Lab D was included, the CVs for the unadjusted values ranged from 74% (serotype 1) to 1048% (serotype 4) with the CVs for most serotypes >200% (Table 5).

Without the data from Lab D, normalization resulted in a decrease in variability for all serotypes except 1 and 23F, although in most cases the reductions were fairly minimal (Table 4). Although there was an increase in variability (indicated as a negative reduction in variability) for serotypes 1 (14% increase) and 23F (1% increase), normalization had a minimal impact on the CVs, increasing from 41% to 46%, and 64% to 65% for serotypes 1 and 23F, respectively. The absolute CVs of the normalized results were <60% for all serotypes except serotypes 4 (79%), 6A (81%), 9V (83%), and 23F (65%). With the inclusion of data from Lab D (Table 5), normalization resulted in significant (>30%) decreases in variability for all serotypes except 1 (3%) and 5 (22%), with the absolute CVs for the normalized data ranging from 64% (serotype 5) to 180% (serotype 4).

The effect of standardization is shown graphically in Fig. 2. For each calibration serum, the unadjusted (y-axis, left panels)

Table 4

Model-based assessment of the effect of normalization, without Lab D data.

	Unadjusted					Normalized					
	Variance components					Variance components					Variability
	Lab	Lab × sample	Run (Lab)	Sample × run (Lab)	%CV	Lab	Lab × sample	Run (Lab)	Sample × run (Lab)	%CV	reduction, %
Pn 1	0.0252	0.0194	0.0217	0.0135	41	0.0340	0.0223	0.0222	0.0144	46	−14
Pn 3	0.0496	0.0187	0.0253	0.0156	55	0.0291	0.0188	0.0226	0.0148	41	22
Pn 4	0.0939	0.0775	0.0515	0.0386	81	0.0892	0.0772	0.0500	0.0383	79	3
Pn 5	0.0519	0.0439	0.0367	0.0310	56	0.0541	0.0426	0.0344	0.0297	58	2
Pn 6A	0.1215	0.0875	0.0533	0.0385	96	0.0909	0.0801	0.0439	0.0371	81	16
Pn 6B	0.0474	0.0398	0.0376	0.0290	54	0.0433	0.0374	0.0330	0.0267	51	9
Pn 7F	0.0433	0.0138	0.0187	0.0113	52	0.0226	0.0127	0.0239	0.0103	39	20
Pn 9V	0.1477	0.0910	0.0645	0.0461	111	0.0967	0.0815	0.0639	0.0463	83	17
Pn 14	0.0265	0.0142	0.0174	0.0103	42	0.0238	0.0140	0.0140	0.0104	38	9
Pn 18C	0.0454	0.0298	0.0303	0.0177	57	0.0330	0.0291	0.0285	0.0176	50	12
Pn 19A	0.0313	0.0165	0.0182	0.0115	42	0.0219	0.0172	0.0128	0.0109	35	19
Pn 19F	0.0203	0.0114	0.0127	0.0082	34	0.0145	0.0118	0.0109	0.0086	30	13
Pn 23F	0.0648	0.0571	0.0360	0.0282	64	0.0662	0.0607	0.0337	0.0280	65	−1

The overall reduction in variability due to normalization is shown for each serotype. Estimates of various variance components and coefficients of variation of the unadjusted and normalized results from the analysis of variance are also shown.

CV = coefficient of variation (expressed as a percent), Lab = variability among the laboratories, Lab × sample = variability associated with the interaction between test sample and laboratory, Pn = pneumococcal serotype, Run (Lab) = variability among runs within each laboratory, Sample × run (Lab) = variability associated with the interaction between test sample and runs within a laboratory.

Table 5

Model-based assessment of the effect of normalization with Lab D data.

	Unadjusted					Normalized					
	Variance components					Variance components					Variability
	Lab	Lab × sample	Run (Lab)	Sample × run (Lab)	%CV	Lab	Lab × sample	Run (Lab)	Sample × run (Lab)	%CV	reduction, %
Pn 1	0.0810	0.0469	0.0379	0.0297	74	0.0662	0.0473	0.0469	0.0295	66	3
Pn 3	0.3503	0.0527	0.0386	0.0264	233	0.0898	0.0527	0.0653	0.0258	79	50
Pn 4	0.8856	0.1877	0.0692	0.0582	1048	0.2695	0.1874	0.0791	0.0581	180	51
Pn 5	0.1131	0.0551	0.0356	0.0295	91	0.0638	0.0543	0.0357	0.0293	64	22
Pn 6A	0.4499	0.1471	0.0704	0.0585	316	0.1863	0.1399	0.1045	0.0581	130	33
Pn 6B	0.3290	0.0897	0.0655	0.0506	218	0.1363	0.0878	0.0659	0.0482	103	37
Pn 7F	0.5714	0.1088	0.0766	0.0698	444	0.1478	0.1082	0.1087	0.0691	109	48
Pn 9V	0.6228	0.2015	0.1225	0.0941	514	0.2585	0.1927	0.1264	0.0920	176	36
Pn 14	0.4827	0.1559	0.1036	0.0773	348	0.2385	0.1537	0.0982	0.0764	162	31
Pn 18C	0.4364	0.1117	0.0925	0.0771	308	0.1626	0.1109	0.1007	0.0768	124	37
Pn 19A	0.3828	0.0902	0.0690	0.0510	259	0.1261	0.0903	0.0649	0.0505	98	44
Pn 19F	0.5761	0.1318	0.0756	0.0671	451	0.1704	0.1316	0.0959	0.0659	123	45
Pn 23F	0.4928	0.1918	0.0406	0.0327	356	0.2299	0.1943	0.0512	0.0323	155	33

The overall reduction in variability due to normalization is shown for each serotype. Estimates of various variance components and coefficients of variation of the unadjusted and normalized results from the analysis of variance are also shown.

CV = coefficient of variation (expressed as a percent), Lab = variability among the laboratories, Lab × sample = variability associated with the interaction between test sample and laboratory, Pn = pneumococcal serotype, Run (Lab) = variability among runs within each laboratory, Sample × run (Lab) = variability associated with the interaction between test sample and runs within a laboratory.

and adjusted (y-axis, right panels) laboratory-specific GMOI as a function of the consensus OI (x-axis) are presented. To improve visualization, the results from different laboratories for each serum are connected by a vertical black line. The effect of normalization can be seen by comparing the length of the vertical lines, with and without normalization. For multiple serotypes, the benefit of normalization was most obvious with high OI sera.

3.3. Determination of consensus OIs for calibration sera

The unadjusted consensus values for the calibration sera for each of the 13 serotypes are shown in Table 6, and the normalized consensus values are shown in Table 7. Due to the disparity of the Lab D results, the consensus values in both tables were estimated based only on the results from Labs A, B, and C. The red, bold text in both tables indicates that at least 1 laboratory reported an irregular result for that sample for that serotype in at least 1 run.

4. Discussion

A critical component for a laboratory to establish an OPA is the ability to determine the performance of the assay with a readily available set of calibration sera. While a panel of calibration sera with consensus opsonic values already exists (FDA Calibration Sera[11]), the number of available vials is extremely limited and the sera are not routinely available. Thus, the first goal of this study was to create a new OPA calibration serum panel that was available to all laboratories. These 20 calibration sera ("Korean OPA Panel A") can be obtained by contacting Dr. Kyung-Hyo Kim at Ewha Womans University (kaykim@ewha.ac.kr) or Dr. Si Hyung Yoo at Biologics Research Division, Ministry of Food and Drug, Republic of Korea (yoosh1130@korea.kr).

In addition, we report consensus OIs for the 13 serotypes included in 13-valent PCV derived from an international collaboration. Table 6 shows unadjusted consensus OIs and Table 7 shows normalized results. Due to the disparate data from Lab D, only data from Labs A, B, and C were used to estimate the consensus values shown in each table (the data from all 4 laboratories can be found in Supplemental Table 1, http://links.lww.com/MD/C217). Although the removal of data from Lab D reduced the number of participating laboratories to 3, some previous 007sp assignments for ELISA were also based on studies involving 3 laboratories.[13,14] An analogous calibration serum panel with assigned values already exists for ELISA use, and rules for determining the comparability of a laboratory's ELISA have been developed (https://www.vaccine.uab.edu/qc3.pdf). The limited amount of data in this study precludes the establishment of such criteria for OPAs. Thus, data from additional laboratories will be needed to construct these parameters and further refine the standardization procedure in the future.

At the moment, the basis for the aberrant results from Lab D is not known. However, as noted in Section 2, Lab D utilized a slower shaking speed than that indicated in the protocol. Although we do not know what effect this change on the results, as noted above, this situation does highlight the need to develop rules for normalizing OPA results based on 007sp, including developing an absolute range of 007sp values that can be used for normalization.

Many reference sera developed for other assays (e.g., ELISA) contain preservatives, such as azide, and/or consist of plasma converted to serum, making them not desirable for OPAs. For instance, some anticoagulants chelate calcium, interfering with phagocytic function. The sera in the Korean OPA Panel A were collected with no preservatives, antibiotics, or anticoagulants and the sera were lyophilized for ease in distribution. In an attempt to obtain samples with low OIs, sera were obtained from 5 adults who were vaccinated 46 to 50 months prior. However, the OIs for these sera (QC01–QC05 in Table 6) were not much different than the OIs of the sera collected 1 month after vaccination (QC06–QC20 in Table 6). The consensus OIs for samples with at least 1 laboratory-reported irregular result are indicated in red, bold font in Tables 6 and 7. Due to the variability associated with such irregular curves, the identified samples should not be used to calibrate the indicated serotype(s), but may be used for other serotypes.

The second goal of this study was to confirm the benefit of normalizing pneumococcal OPA results using reference serum

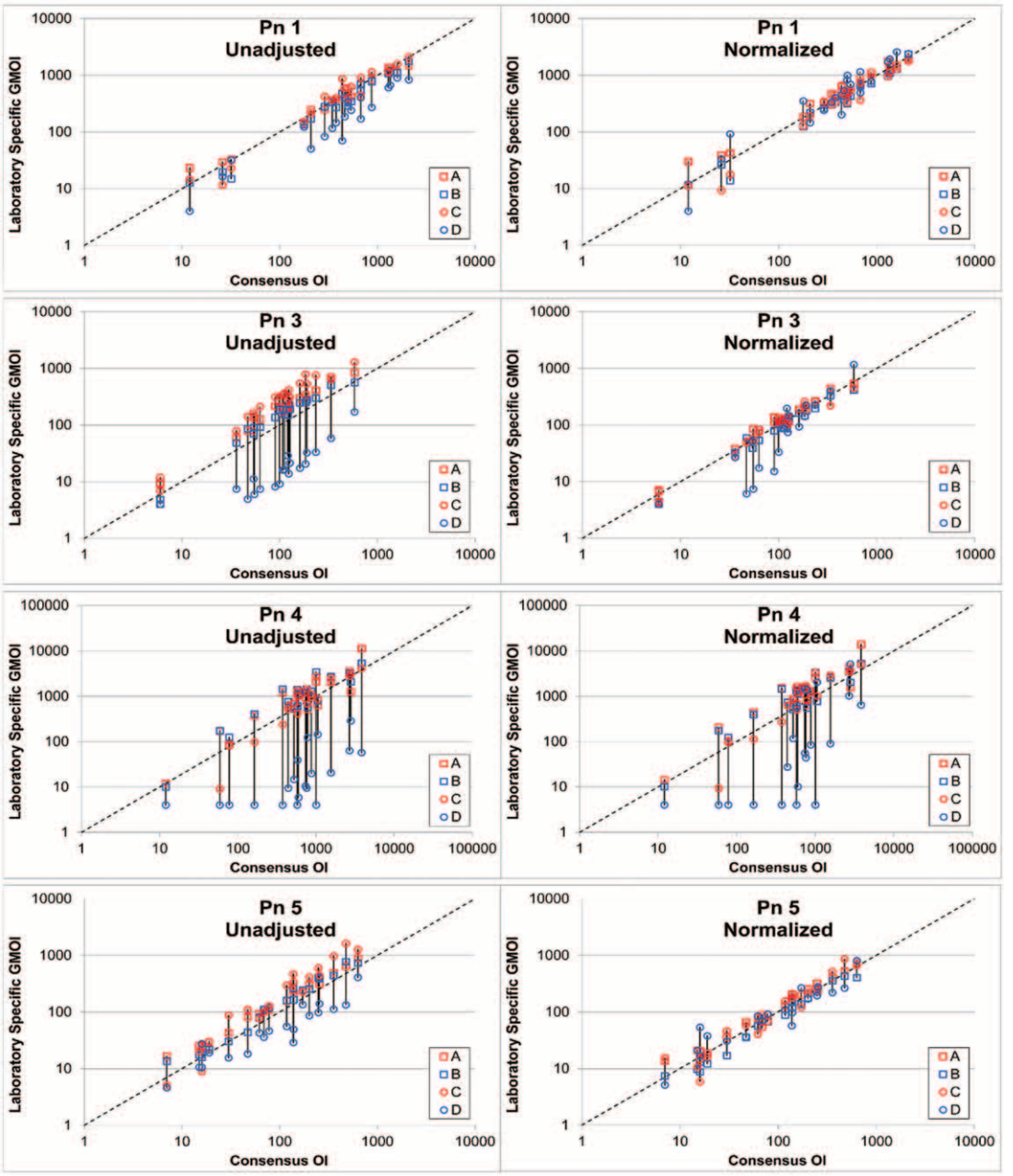

Figure 2. Effect of normalization. The GMOIs obtained by each laboratory (GMOIs, y-axis) as a function of the consensus OI (x-axis) is shown for each of the 20 sera. For each serotype, the left panel displays the unadjusted data and the right panel shows the normalized data. Each plot also has a line of identity (dashed line). The consensus OI includes data from Lab D. GMOI = geometric mean opsonic index, OI = opsonic index, Pn = pneumococcal serotype.

007sp.[11] Indeed, normalization of the results from Labs A, B, and C reduced the variability for 10 of the 13 target serotypes, but the reductions were modest for many serotypes (Table 4) largely because the unadjusted results agreed well among the 3 laboratories even before the normalization. When the data from Lab D was included (Table 5), normalization significantly reduced the deviation of Lab D's results from the consensus values similar to the previous study.[11] Taken together, our results confirm that normalization would significantly reduce interlaboratory variability.

In the previous study,[11] the absolute 007sp results obtained by the 6 individual laboratories were relatively comparable, with most values differing from the assigned values by <3-fold. In the current study, 007sp results for Labs A, B, and C were also within 3-fold of the assigned values, but the 007sp results from Lab D differed by more than 3-fold for most of the serotypes tested. In fact, the 007sp results for all serotypes were lower than the assigned values, indicating the OPA for Lab D is less sensitive than that of the other laboratories. Although normalization reduced the variability between the 4 laboratories (see Table 5),

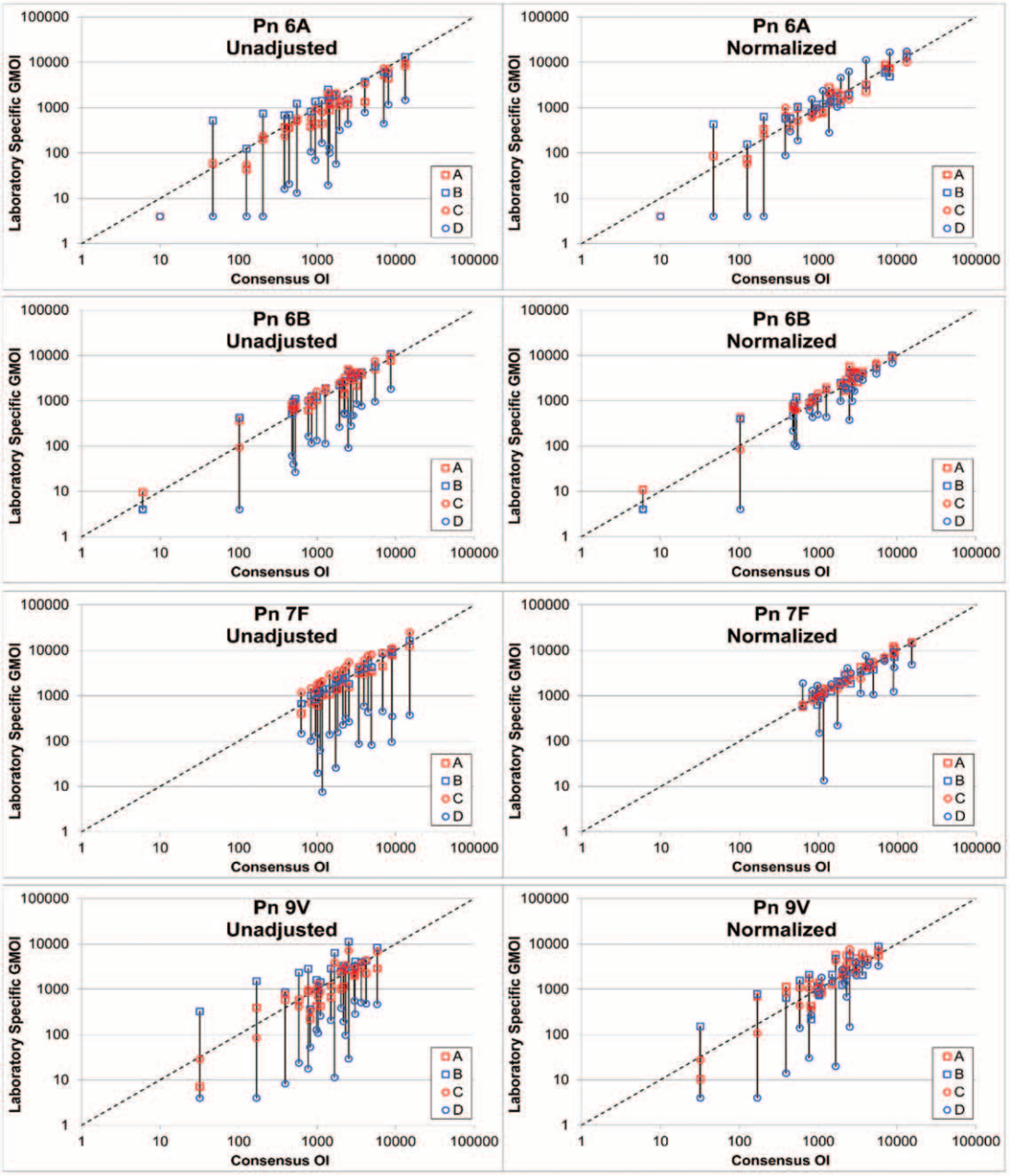

Figure 2. (Continued).

the absolute variability remained high for many serotypes after normalization, with most CVs >100% (Table 5). Based on these results and the results of the previous study, we tentatively propose that a laboratory's absolute 007sp result for a serotype must be within 3-fold of the assigned value (indicating an assay sensitivity comparable to others) to be used for normalizing OPA data.

It is worth noting that no criteria for OPA sensitivity could be developed until 007sp with assigned values became available. However, to reap the full benefits of OPA standardization, additional operation rules for implementation of standardization still need to be developed. For example, the optimum number of 007sp results used to normalize a sample must be determined. In this study as well as the previous study, normalization was based on a single 007sp result within a run. If the 007sp result was incorrect due to random or technical errors, results of the entire run would be affected. Thus, we believe that 007sp should be, in the future, analyzed more than once, perhaps 3 times per run, and the average of the 3 results should be used to normalize the data from the entire run. Also, as mentioned above, parameters to better define a "calibrated" assay must be developed.

In summary, we have created and characterized a panel of sera that can be used to determine the comparability of a laboratory's OPA results to the results from other laboratories. Now, with this newly available calibration serum panel,

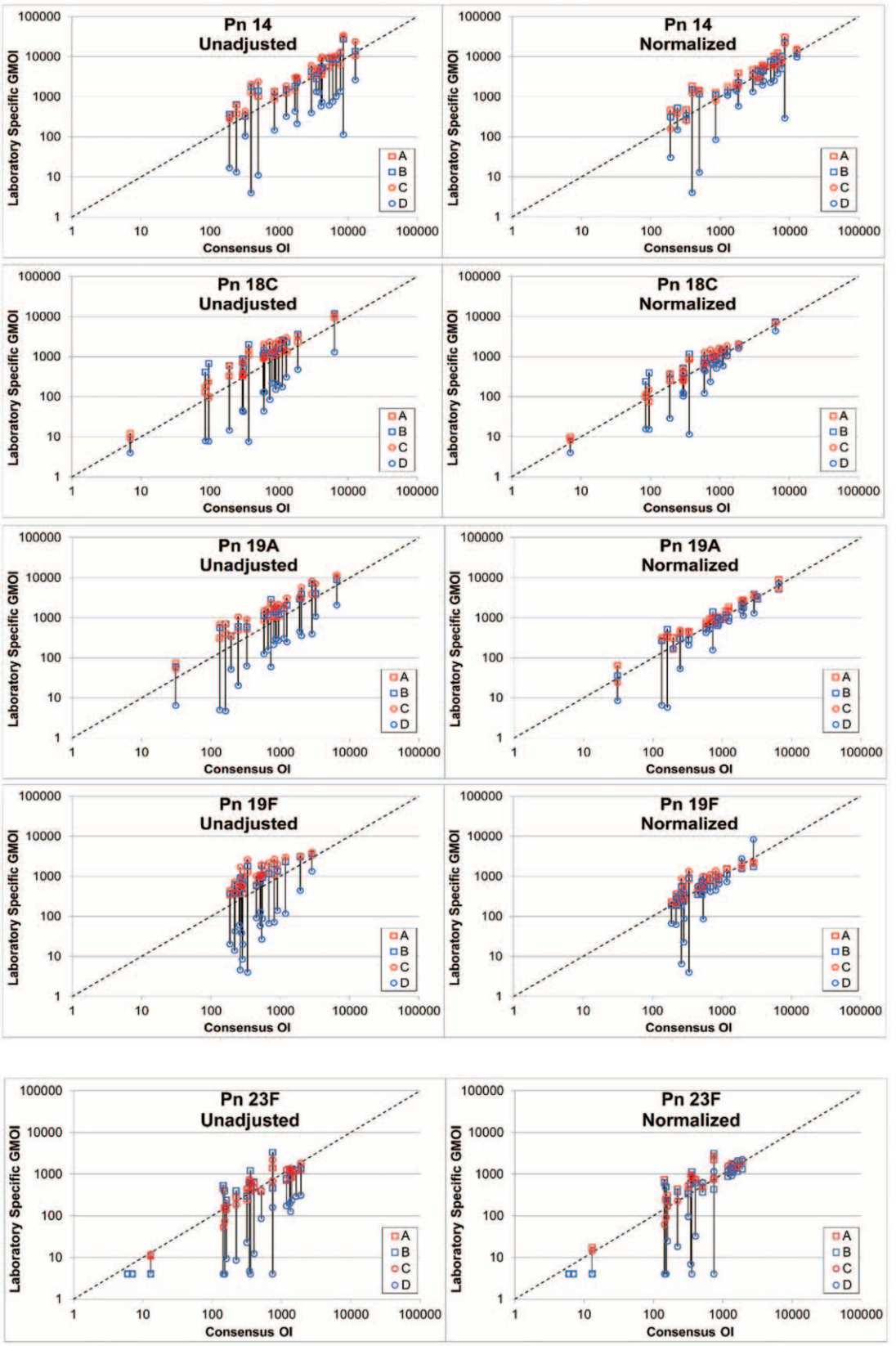

Figure 2. (Continued).

Table 6

Unadjusted calibration sera consensus OIs (without Lab D).

		Pn 1	Pn 3	Pn 4	Pn 5	Pn 6A	Pn 6B	Pn 7F
QC-01	Consensus OI	505	846	96	318	2541	2327	2194
	(95% CI)	(355, 720)	(302, 2365)	(52, 176)	(173, 581)	(603, 10,705)	(727, 7450)	(665, 7235)
QC-02	Consensus OI	206	**99**	753	106	**46**	**5**	2799
	(95% CI)	(123, 347)	**(39, 248)**	(63, 8987)	(90, 125)	**(0, 17,915)**	**(2, 19)**	(1044, 7502)
QC-03	Consensus OI	22	97	**8**	10	822	1071	933
	(95% CI)	(7, 71)	(29, 321)	**(2, 37)**	(2, 52)	(196, 3451)	(507, 2265)	(420, 2076)
QC-04	Consensus OI	352	8	1119	939	**4**	244	1093
	(95% CI)	(296, 417)	(2, 31)	(766, 1635)	(459, 1918)	**(NA)**	(30, 1981)	(274, 4365)
QC-05	Consensus OI	16	7	**63**	22	1279	1250	687
	(95% CI)	(6, 46)	(3, 16)	**(1, 4730)**	(10, 49)	(913, 1792)	(697, 2244)	(180, 2617)
QC-06	Consensus OI	1207	208	1345	194	1391	3214	2491
	(95% CI)	(899, 1620)	(71, 612)	(1033, 1752)	(76, 496)	(1012, 1912)	(1334, 7747)	(452, 13,732)
QC-07	Consensus OI	1777	344	2718	594	1153	2411	7026
	(95% CI)	(1011, 3124)	(136, 871)	(1453, 5087)	(199, 1776)	(638, 2083)	(1600, 3635)	(2685, 18,386)
QC-08	Consensus OI	309	231	1280	21	10,300	9503	1442
	(95% CI)	(156, 612)	(113, 469)	(965, 1699)	(12, 37)	(5635, 18,827)	(5579, 16,187)	(535, 3888)
QC-09	Consensus OI	19	340	2020	73	563	728	9286
	(95% CI)	(5, 67)	(120, 961)	(646, 6313)	(21, 261)	(219, 1447)	(392, 1353)	(5802, 14,860)
QC-10	Consensus OI	1183	221	762	48	795	3288	4176
	(95% CI)	(842, 1662)	(72, 679)	(488, 1191)	(13, 186)	(180, 3515)	(2242, 4821)	(1717, 10,157)
QC-11	Consensus OI	1262	61	581	83	443	661	1010
	(95% CI)	(764, 2085)	(30, 125)	(354, 953)	(27, 254)	(162, 1213)	(342, 1275)	(414, 2460)
QC-12	Consensus OI	454	219	6296	456	329	1824	2593
	(95% CI)	(248, 830)	(83, 575)	(1713, 23,135)	(254, 816)	(57, 1917)	(1223, 2722)	(1330, 5056)
QC-13	Consensus OI	910	269	1103	927	2061	2267	9921
	(95% CI)	(548, 1510)	(103, 702)	(678, 1796)	(268, 3211)	(1213, 3504)	(1608, 3195)	(6892, 14,283)
QC-14	Consensus OI	338	604	522	16	6168	4028	3730
	(95% CI)	(204, 559)	(401, 911)	(312, 872)	(4, 62)	(3983, 9551)	(3014, 5383)	(2405, 5787)
QC-15	Consensus OI	581	406	3178	311	696	876	1917
	(95% CI)	(245, 1381)	(92, 1791)	(2234, 4522)	(126, 765)	(197, 2462)	(476, 1612)	(894, 4108)
QC-16	Consensus OI	450	453	2414	229	1722	3774	4842
	(95% CI)	(194, 1041)	(140, 1464)	(1658, 3516)	(93, 563)	(842, 3522)	(2614, 5450)	(1464, 16,014)
QC-17	Consensus OI	758	121	637	118	66	851	1336
	(95% CI)	(455, 1261)	(62, 233)	(364, 1117)	(96, 146)	(17, 259)	(429, 1689)	(611, 2922)
QC-18	Consensus OI	142	203	623	25	5594	6035	17,293
	(95% CI)	(120, 168)	(87, 471)	(355, 1094)	(11, 56)	(2929, 10,681)	(3246, 11,219)	(6555, 45,624)
QC-19	Consensus OI	358	233	244	377	390	4728	1643
	(95% CI)	(276, 464)	(122, 443)	(34, 1748)	(233, 609)	(83, 1835)	(2927, 7639)	(452, 5968)
QC-20	Consensus OI	422	134	1149	238	1274	3457	5187
	(95% CI)	(203, 877)	(46, 395)	(819, 1613)	(153, 370)	(798, 2034)	(2540, 4704)	(1974, 13,624)

		Pn 9V	Pn 14	Pn 18C	Pn 19A	Pn 19F	Pn 23F
QC-01	Consensus OI	880	5726	1271	1777	1012	1084
	(95% CI)	(168, 4601)	(1539, 21,298)	(861, 1876)	(1343, 2352)	(735, 1392)	(671, 1749)
QC-02	Consensus OI	369	4414	489	6136	1576	4
	(95% CI)	(9, 15,108)	(2018, 9650)	(212, 1132)	(2271, 16,579)	(703, 3531)	(NA)
QC-03	Consensus OI	983	528	1688	2381	380	889
	(95% CI)	(455, 2123)	(236, 1180)	(650, 4386)	(1286, 4407)	(334, 432)	(406, 1944)
QC-04	Consensus OI	40	1187	587	682	561	4
	(95% CI)	(0, 4927)	(566, 2489)	(214, 1611)	(256, 1814)	(252, 1251)	(NA)
QC-05	Consensus OI	736	362	215	653	412	317
	(95% CI)	(413, 1313)	(228, 572)	(49, 936)	(313, 1362)	(255, 664)	(152, 663)
QC-06	Consensus OI	2388	4188	1283	1444	714	1050
	(95% CI)	(1250, 4560)	(2741, 6401)	(853, 1928)	(658, 3169)	(305, 1669)	(588, 1874)
QC-07	Consensus OI	5403	10,176	248	1426	1548	777
	(95% CI)	(1347, 21,678)	(3835, 27,000)	(22, 2834)	(567, 3588)	(846, 2832)	(298, 2026)
QC-08	Consensus OI	3491	1539	1484	1928	1081	2159
	(95% CI)	(1356, 8986)	(952, 2489)	(557, 3950)	(523, 7113)	(406, 2875)	(729, 6397)
QC-09	Consensus OI	1805	1508	**10**	584	1808	562
	(95% CI)	(526, 6200)	(523, 4352)	**(0, 4093)**	(261, 1306)	(582, 5619)	(235, 1345)
QC-10	Consensus OI	809	1692	574	498	392	1203
	(95% CI)	(171, 3824)	(847, 3379)	(155, 2127)	(178, 1395)	(277, 555)	(992, 1458)

(*continued*)

Table 6

(continued).

		Pn 9V	Pn 14	Pn 18C	Pn 19A	Pn 19F	Pn 23F
QC-11	Consensus OI	1309	3637	1649	10,222	815	526
	(95% CI)	(206, 8329)	(1743, 7590)	(873, 3114)	(7422, 14,078)	(508, 1308)	(299, 926)
QC-12	Consensus OI	2219	15,304	2090	1136	1474	273
	(95% CI)	(540, 9127)	(5178, 45,236)	(742, 5890)	(555, 2324)	(635, 3425)	(104, 714)
QC-13	Consensus OI	1353	8639	2114	4093	2543	235
	(95% CI)	(274, 6686)	(6169, 12,100)	(965, 4632)	(1919, 8730)	(1736, 3725)	(10, 5782)
QC-14	Consensus OI	3589	5925	10,763	3104	3130	1256
	(95% CI)	(2572, 5008)	(2310, 15,194)	(7306, 15,857)	(2585, 3726)	(2624, 3734)	(941, 1678)
QC-15	Consensus OI	4180	2754	1474	1286	906	**187**
	(95% CI)	(1450, 12,054)	(1732, 4380)	(599, 3624)	(696, 2375)	(604, 1358)	**(10, 3472)**
QC-16	Consensus OI	5958	30,307	1499	4777	1829	570
	(95% CI)	(962, 36,905)	(20,325, 45,191)	(683, 3294)	(2084, 10,948)	(756, 4425)	(358, 907)
QC-17	Consensus OI	295	2053	422	354	505	182
	(95% CI)	(154, 567)	(842, 5006)	(238, 748)	(330, 380)	(265, 966)	(82, 403)
QC-18	Consensus OI	3090	9275	3047	1421	1058	1475
	(95% CI)	(2284, 4181)	(6650, 12,935)	(1617, 5741)	(648, 3116)	(945, 1185)	(829, 2622)
QC-19	Consensus OI	825	321	1074	**61**	530	392
	(95% CI)	(82, 8348)	(167, 618)	(756, 1526)	**(30, 127)**	(306, 917)	(344, 447)
QC-20	Consensus OI	3142	7898	976	1418	3678)	8
	(95% CI)	(1619, 6097)	(3385, 18,424)	(670, 1423)	(868, 2317)	(3025, 4473)	(2, 35)

For each serum in the panel, the consensus OIs and the 95% CI are shown for the indicated serotypes. Results in red text indicate at least 1 laboratory reported an irregular result for at least 1 run. CI = confidence interval, NA = not applicable (all reported values were undetectable and/or irregular), OI = opsonic index, Pn = pneumococcal serotype.

Table 7

Normalized calibration sera consensus OIs (without Lab D).

		Pn 1	Pn 3	Pn 4	Pn 5	Pn 6A	Pn 6B	Pn 7F
QC-01	Consensus OI	566	463	108	213	2883	2300	1827
	(95% CI)	(217, 1475)	(329, 652)	(80, 145)	(133, 342)	(1663, 4999)	(1052, 5026)	(1227, 2719)
QC-02	Consensus OI	236	**52**	859	68	**57**	**5**	2458
	(95% CI)	(122, 455)	**(34, 80)**	(82, 9041)	(34, 137)	**(0, 14,744)**	**(1, 30)**	(1754, 3445)
QC-03	Consensus OI	22	48	**9**	6	933	1064	844
	(95% CI)	(5, 93)	(28, 82)	**(2, 44)**	(1, 50)	(532, 1634)	(670, 1689)	(418, 1704)
QC-04	Consensus OI	351	4	1276	584	**4**	246	960
	(95% CI)	(188, 653)	(1, 13)	(1015, 1604)	(262, 1300)	**(NA)**	(27, 2265)	(588, 1566)
QC-05	Consensus OI	16	3	**72**	14	1451	1242	604
	(95% CI)	(4, 63)	(1, 12)	**(1, 4776)**	(4, 42)	(822, 2561)	(877, 1758)	(380, 959)
QC-06	Consensus OI	1204	105	1511	121	1802	3177	2259
	(95% CI)	(751, 1930)	(53, 211)	(1122, 2033)	(58, 251)	(1017, 3192)	(1865, 5412)	(1195, 4268)
QC-07	Consensus OI	2006	181	3053	420	1379	2383	6686
	(95% CI)	(1289, 3122)	(140, 233)	(1991, 4679)	(262, 673)	(987, 1929)	(1746, 3254)	(5486, 8150)
QC-08	Consensus OI	308	117	1438	13	12,312	9392	1201
	(95% CI)	(198, 479)	(75, 182)	(1119, 1847)	(5, 37)	(7428, 20,408)	(7122, 12,384)	(780, 1850)
QC-09	Consensus OI	21	179	2269	52	677	720	8644
	(95% CI)	(3, 153)	(112, 287)	(712, 7232)	(19, 141)	(452, 1015)	(459, 1129)	(5031, 14,854)
QC-10	Consensus OI	1180	112	841	30	902	3079	3969
	(95% CI)	(493, 2823)	(68, 185)	(524, 1351)	(8, 111)	(449, 1812)	(2011, 4715)	(2914, 5405)
QC-11	Consensus OI	1362	32	652	56	503	619	874
	(95% CI)	(827, 2242)	(20, 54)	(370, 1151)	(18, 177)	(216, 1173)	(327, 1169)	(552, 1382)
QC-12	Consensus OI	441	108	7105	271	374	1803	2254
	(95% CI)	(294, 663)	(77, 150)	(1694, 29,798)	(176, 416)	(114, 1221)	(1223, 2659)	(1242, 4091)
QC-13	Consensus OI	907	136	1326	580	2339	2407	9645
	(95% CI)	(498, 1655)	(93, 200)	(755, 2329)	(241, 1397)	(1299, 4212)	(1745, 3320)	(5145, 18,081)
QC-14	Consensus OI	372	318	589	11	7290	3981	3242
	(95% CI)	(227, 607)	(131, 770)	(390, 890)	(2, 74)	(4217, 12,602)	(3135, 5055)	(1336, 7868)
QC-15	Consensus OI	565	201	3729	184	790	922	1777
	(95% CI)	(309, 1034)	(97, 419)	(2643, 5260)	(109, 313)	(270, 2307)	(383, 2221)	(990, 3189)
QC-16	Consensus OI	495	238	2665	156	2035	3730	4602
	(95% CI)	(333, 737)	(155, 366)	(1859, 3819)	(90, 273)	(1274, 3251)	(2691, 5170)	(2327, 9100)

(*continued*)

Table 7

(continued).

		Pn 1	Pn 3	Pn 4	Pn 5	Pn 6A	Pn 6B	Pn 7F
QC-17	Consensus OI	756	61	716	74	83	841	1157
	(95% CI)	(468, 1220)	(30, 125)	(319, 1605)	(47, 116)	(24, 286)	(584, 1212)	(684, 1956)
QC-18	Consensus OI	142	103	700	15	6349	5964	14,734
	(95% CI)	(83, 243)	(56, 188)	(478, 1025)	(8, 31)	(3469, 11,620)	(3676, 9677)	(9111, 23,829)
QC-19	Consensus OI	400	121	274	248	443	4673	1428
	(95% CI)	(238, 671)	(82, 179)	(45, 1693)	(203, 305)	(98, 2007)	(2829, 7718)	(808, 2524)
QC-20	Consensus OI	421	68	1291	149	1446	3417	4508
	(95% CI)	(220, 806)	(39, 117)	(802, 2076)	(74, 301)	(771, 2713)	(1914, 6100)	(2687, 7565)

		Pn 9V	Pn 14	Pn 18C	Pn 19A	Pn 19F	Pn 23F
QC-01	Consensus OI	1017	5029	834	1170	555	1326
	(95% CI)	(618, 1674)	(3250, 7783)	(533, 1304)	(624, 2194)	(348, 884)	(931, 1890)
QC-02	Consensus OI	388	3855	330	3720	879	4
	(95% CI)	(23, 6430)	(2176, 6830)	(189, 575)	(2911, 4754)	(522, 1481)	(NA)
QC-03	Consensus OI	1136	471	1126	1428	208	1154
	(95% CI)	(442, 2916)	(275, 807)	(454, 2792)	(642, 3174)	(156, 279)	(557, 2390)
QC-04	Consensus OI	42	1037	388	399	312	4
	(95% CI)	(2, 869)	(539, 1996)	(190, 791)	(206, 773)	(190, 511)	(NA)
QC-05	Consensus OI	850	316	141	382	226	412
	(95% CI)	(414, 1747)	(128, 777)	(43, 456)	(204, 717)	(142, 359)	(224, 759)
QC-06	Consensus OI	2733	3679	908	844	420	1285
	(95% CI)	(1306, 5719)	(1846, 7330)	(647, 1275)	(577, 1236)	(220, 801)	(886, 1864)
QC-07	Consensus OI	7029	8938	163	957	911	951
	(95% CI)	(3981, 12,411)	(6146, 12,998)	(20, 1346)	(791, 1158)	(643, 1291)	(621, 1458)
QC-08	Consensus OI	4541	1352	1057	1128	636	2643
	(95% CI)	(3220, 6404)	(718, 2547)	(493, 2266)	(672, 1893)	(316, 1282)	(1701, 4106)
QC-09	Consensus OI	1834	1325	**7**	392	999	688
	(95% CI)	(773, 4354)	(955, 1837)	**(0, 3451)**	(208, 737)	(439, 2274)	(363, 1303)
QC-10	Consensus OI	934	1486	376	287	215	1489
	(95% CI)	(600, 1455)	(868, 2543)	(143, 991)	(223, 369)	(153, 301)	(806, 2752)
QC-11	Consensus OI	1512	3195	1082	6355	447	644
	(95% CI)	(718, 3187)	(1220, 8369)	(597, 1959)	(2918, 13,839)	(236, 845)	(266, 1557)
QC-12	Consensus OI	2534	13,646	1372	641	808	334
	(95% CI)	(1648, 3897)	(8759, 21,257)	(629, 2989)	(410, 1002)	(344, 1901)	(141, 788)
QC-13	Consensus OI	1545	8030	1387	2358	1394	310
	(95% CI)	(628, 3799)	(3593, 17,949)	(847, 2272)	(1358, 4094)	(853, 2278)	(11, 8749)
QC-14	Consensus OI	3655	5204	7225	2056	1730	1538
	(95% CI)	(928, 14,397)	(3197, 8471)	(4846, 10,773)	(1050, 4025)	(1258, 2380)	(954, 2478)
QC-15	Consensus OI	4817	2509	967	725	496	**258**
	(95% CI)	(2506, 9259)	(929, 6780)	(511, 1829)	(377, 1394)	(291, 847)	**(14, 4850)**
QC-16	Consensus OI	6068	24,471	1006	3174	1011	697
	(95% CI)	(3649, 10,091)	(14,373, 41,662)	(503, 2014)	(2380, 4233)	(535, 1908)	(484, 1004)
QC-17	Consensus OI	325	1803	295	204	291	223
	(95% CI)	(139, 764)	(1213, 2680)	(164, 528)	(77, 539)	(163, 519)	(108, 459)
QC-18	Consensus OI	3452	8147	1970	819	575	1805
	(95% CI)	(969, 12,303)	(3168, 20,949)	(1412, 2749)	(655, 1023)	(388, 851)	(904, 3603)
QC-19	Consensus OI	922	282	694	**38**	288	480
	(95% CI)	(179, 4748)	(73, 1086)	(539, 894)	**(11, 136)**	(156, 530)	(255, 904)
QC-20	Consensus OI	3510	6937	631	817	1997	10
	(95% CI)	(1713, 7191)	(4081, 11,792)	(418, 952)	(434, 1537)	(1436, 2777)	(1, 79)

For each serum in the panel, the consensus OI and the 95% CI are shown for the indicated serotypes. Results in red text indicate at least 1 laboratory reported an irregular result for at least 1 run. CI = confidence interval, NA = not applicable (all reported values were undetectable and/or irregular), OI = opsonic index, Pn = pneumococcal serotype.

individual laboratories can better characterize and standardize their OPAs, making the assay an even more powerful tool in vaccine evaluation.

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Author contributions

Conceptualization: Robert L Burton, Moon H Nahm, Kyung-Hyo Kim.

Data curation: Robert L Burton, Han Wool Kim, Soyoung Lee, Jee-Hyun Seok, Anne Balloch, Paul Licciardi, Rachel Marimla, Sejong Bae, Kyung-Hyo Kim.
Formal analysis: Robert L Burton, Sejong Bae, Kyung-Hyo Kim.
Funding acquisition: Moon H Nahm, Kyung-Hyo Kim.
Investigation: Robert L Burton, Han Wool Kim, Soyoung Lee, Jee-Hyun Seok, Sang Heon Lee, Anne Balloch, Paul Licciardi, Rachel Marimla, Sejong Bae.
Methodology: Robert L Burton, Sejong Bae, Moon H Nahm, Kyung-Hyo Kim.
Project administration: Robert L Burton, Kyung-Hyo Kim.
Resources: Robert L Burton, Han Wool Kim, Soyoung Lee, Hun Kim, Sang Heon Lee, Anne Balloch, Moon H Nahm, Kyung-Hyo Kim.
Software: Robert L Burton, Sejong Bae, Moon H Nahm.
Supervision: Robert L Burton, Moon H Nahm, Kyung-Hyo Kim.
Validation: Robert L Burton, Sejong Bae, Moon H Nahm.
Visualization: Robert L Burton, Sejong Bae.
Writing – original draft: Robert L Burton, Sejong Bae, Moon H Nahm, Kyung-Hyo Kim.
Writing – review & editing: Robert L Burton, Han Wool Kim, Soyoung Lee, Hun Kim, Jee-Hyun Seok, Sang Heon Lee, Anne Balloch, Paul Licciardi, Rachel Marimla, Sejong Bae, Moon H Nahm, Kyung-Hyo Kim.

References

[1] Yildirim I, Shea KM, Pelton SI. Pneumococcal disease in the era of pneumococcal conjugate vaccine. Infect Dis Clin North Am 2015;29:679–97.
[2] Weinberger DM, Malley R, Lipsitch M. Serotype replacement in disease after pneumococcal vaccination. Lancet 2011;378: 1962–73.
[3] Jodar L, Butler JC, Carlone G, et al. Serological criteria for evaluation and licensure of pneumococcal conjugate vaccine formulations for use in infants. Vaccine 2003;21:3265–72.
[4] Lee H, Nahm MH, Kim KH. The effect of age on the response to the pneumococcal polysaccharide vaccine. BMC Infect Dis 2010;10:60.
[5] Yu X, Gray B, Chang SJ, et al. Immunity to cross-reactive serotypes induced by pneumococcal conjugate vaccines in infants. J Infect Dis 1999;180:1569–76.
[6] Lee H, Nahm MH, Burton R, et al. Immune response in infants to the heptavalent pneumococcal conjugate vaccine against vaccine-related serotypes 6A and 19A. Clin Vaccine Immunol 2009;16:376–81.
[7] Hu BT, Yu X, Jones TR, et al. Approach to validating an opsonophagocytic assay for *Streptococcus pneumoniae*. Clin Diagn Lab Immunol 2005;12:287–95.
[8] Burton RL, Nahm MH. Development and validation of a fourfold multiplexed opsonization assay (MOPA4) for pneumococcal antibodies. Clin Vaccine Immunol 2006;13:1004–9.
[9] Henckaerts I, Durant N, De Grave D, et al. Validation of a routine opsonophagocytosis assay to predict invasive pneumococcal disease efficacy of conjugate vaccine in children. Vaccine 2007;25:2518–27.
[10] Rose CE, Romero-Steiner S, Burton RL, et al. Multilaboratory comparison of Streptococcus pneumoniae opsonophagocytic killing assays and their level of agreement for the determination of functional antibody activity in human reference sera. Clin Vaccine Immunol 2011;18:135–42.
[11] Burton RL, Antonello J, Cooper D, et al. Assignment of opsonic values to pneumococcal reference serum 007sp for use in opsonophagocytic assays for 13 serotypes. Clin Vaccine Immunol 2017;24:e00457–516.
[12] Goldblatt D, Plikaytis BD, Akkoyunlu M, et al. Establishment of a new human pneumococcal standard reference serum, 007sp. Clin Vaccine Immunol 2011;18:1728–36.
[13] Goldblatt D, Tan CY, Burbidge P, et al. Assignment of weight-based antibody units for seven additional serotypes to a human pneumococcal standard reference serum, 007sp. Clin Vaccine Immunol 2015;22:1154–9.
[14] Goldblatt D, McKeen A, Burbidge P, et al. Assignment of weight-based antibody units for four additional serotypes to a human anti-pneumococcal standard reference serum 007sp. Clin Vaccine Immunol 2017;24:e00194–217.