

007sp OPA Standardization Manuscript

ASSIGNMENT OF OPSONIC VALUES TO PNEUMOCOCCAL REFERENCE SERUM 007SP FOR USE IN OPSONOPHAGOCYtic ASSAYS FOR 13 SEROTYPES

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Abstract (248 words)

Opsonophagocytic assays (OPAs) are routinely used for assessing the immunogenicity of pneumococcal vaccines, with OPA data often utilized for licensure of new vaccine formulations. However, no reference serum for pneumococcal OPAs is available, making evaluation of data among different laboratories difficult. This international collaboration was initiated to: 1) assign consensus opsonic indexes (OIs) to Pneumococcal Reference Serum Lot 007sp (“007sp”) and a panel of calibration sera; and 2) determine if normalization with 007sp decreases the OPA variability among laboratories.

To meet these goals, six participating laboratories tested a panel of sera in five runs for 13 serotypes. For each serum, consensus OIs were obtained using a mixed effects ANOVA model. For the calibration sera, normalized consensus values were also determined based on 007sp.

For each serotype, the overall reduction in inter-laboratory variability was calculated by comparing the coefficients of variation of the unadjusted and the normalized values. Normalization of the results substantially reduced the inter-laboratory variability, ranging from a 15% reduction in variability for serotype 9V to 64% for serotype 7F. Normalization also increased the proportion of data within 2-fold of the consensus value from approximately 70% (average of all serotypes) to >90%.

Based on the data obtained in this study, Pneumococcal Reference Standard Lot 007sp will likely be a useful reagent for normalizing pneumococcal OPA results from different laboratories. The data also support the use of the 16 FDA OPA calibrations sera as part of the initial evaluation of new assays or periodic assessment of established assays.

INTRODUCTION

Conjugate vaccines targeting the capsular polysaccharides (PSs) of *Streptococcus pneumoniae* have been largely successful in reducing the incidence of invasive pneumococcal disease caused by vaccine serotypes in various countries throughout the world, in both children and adults (reviewed in (1)). Although large clinical trials were performed to demonstrate the efficacy of the first pneumococcal conjugate vaccine, such efficacy trials are not practical for the evaluation of new generation vaccines with increased serotypic coverage. Therefore, surrogate markers of protection have been used to evaluate new vaccine formulations.

Comparison of the immunogenicity of a prospective vaccine to that of the current vaccine in terms of serotype-specific IgG concentrations using ELISA has been utilized in assessing the efficacy of candidate vaccines in pediatric populations. Since a correlation between serum antibody concentrations and vaccine efficacy has been established only for this age group (2), the use of ELISA for the evaluation of vaccine efficacy is restricted to pediatric populations. Although the development of a third generation ELISA (reviewed in (3)) has increased the specificity of the assay, serum antibody concentrations measured in ELISA may not always reflect their functional capacity, especially in adults (4, 5). Therefore, ELISA data is not accepted for licensure of pneumococcal vaccines in adults.

In vivo, antibodies against pneumococcal capsular PS are thought to function by opsonizing the bacteria for subsequent phagocytosis and killing by granulocytes (6). Thus, *in vitro* opsonophagocytic killing assays (OPAs) were developed to mimic this mechanism (7-9). Multiple improvements have since been made to the assay, resulting in various assay protocols (10, 11), including a multiplexed format (MOPA) (12), that are suitable for vaccine studies. Consequently, the OPA has become an important tool for evaluating the immunogenicity of new pneumococcal vaccines, particularly in adults.

A previous international inter-laboratory OPA study (13) showed a reasonably good correlation between results obtained by different labs that utilize different assays formats. However, the absolute agreement of the results was less than desirable with a 2-fold range above and below the consensus values (i.e., a 4-fold range from the upper limit to the lower limit) encompassing approximately 68% of the results. A 4-fold range above and below the consensus value (i.e., a 16-fold range from upper limit to lower limit) was needed to capture approximately 90% of the results. The study authors concluded that OPAs may need additional control and absolute OPA results were not necessarily comparable among different laboratories.

When the new pneumococcal ELISA reference serum, 007sp, was produced, it was intended to serve as a reference serum for both ELISAs and OPAs (14). When sera were pooled to create 007sp, sixteen single-donor sera were prepared for OPA calibration purposes (14). In 2012, an international collaborative study involving 6 laboratories familiar with OPAs was devised to: 1) assign consensus values to 007sp and a panel of 16 sera to be used for calibrating OPAs; and 2) determine if normalization of results with 007sp had any impact on the inter-laboratory agreement of OPA results. Herein described are the results of the collaboration.

MATERIALS AND METHODS

Participating laboratories. The locations and the formats used by each of the six laboratories selected to participate are indicated in Table 1. Note, the laboratories are listed alphabetically in Table 1 and this order is not associated with the letter designations (A-F) utilized throughout this manuscript. Participation in the study was limited to laboratories with substantial experience with pneumococcal OPAs that were routinely performing the assays.

Sera. The production of 007sp and the 16 FDA OPA calibration sera (samples 007A through 007P) have been described previously (14).

Preliminary data (data not shown) indicated that for many serotypes, the calibration sera panel lacked samples with relatively low opsonization indexes (OIs, defined below). Four unvaccinated sera (15) that were included in those preliminary runs to serve as low OI samples produced irregular killing curves for multiple serotypes, and thus were not useful for this purpose. So that samples with low OIs could be included in this evaluation, four low OI samples were prepared by spiking a calibration serum into an immunoglobulin-depleted serum (IDS). The IDS purchased from BBI Solutions (Cardiff, UK) possessed some residual opsonic activity against some serotypes. Additional IgM depletion was performed using affinity chromatography (16) until the residual opsonic activity was removed. One set of four sera was prepared for serotypes 4, 6B, 14, and 19A (sera S17, S18, S19, and S20); and a second set of four sera was prepared for the remaining 9 serotypes (sera S21, S22, S23, and S24).

Study design. For the 13 target serotypes, each participating laboratory tested 21 sera (007sp, 16 FDA OPA calibration sera, and four prepared low-OI sera described above) in 5 separate runs. 007sp was included on every assay plate of every run (except lab E which included 007sp at least once in each run, but not on each plate). For each sample, the OI calculated by the participating laboratory was used for statistical analyses.

OPAs. All laboratories tested samples for serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F except laboratory E which did not test serotype 19A. The published reference for the OPA used by each participating laboratory is shown in Table 1. All participating laboratories utilized a killing-type OPA with frozen aliquots of bacteria as targets, baby rabbit serum as a complement source, and differentiated HL60 cells as effectors. The target strains were the same for all laboratories utilizing the MOPA, but were different for each of the laboratories using the singleplex format.

For each result, the OI was calculated and tabulated by the participating laboratory. Although different algorithms were utilized to determine the OI, all laboratories defined an OI as the estimated dilution of serum that kills 50% of the target bacteria. Control wells (defining 0% killing) containing target bacteria, complement, and HL60 cells (no test serum) were included in every assay. Results that failed to meet a laboratory's assay system suitability criteria were indicated as "Indeterminable" (Table 2 indicates the numbers and sources of indeterminable results for the calibration sera).

Statistical analyses. Within each laboratory, results for each serum were screened for outliers, with outliers defined as OIs differing by more than 4-fold from the respective laboratory-specific median OI. Results identified as intra-laboratory outliers (see Table 2) were removed prior to analysis.

To estimate the 007sp consensus OI for each serotype, the log transformed OIs for 007sp were fit using a mixed effects ANOVA model containing the random terms Lab, Run(Lab) and Plate(RunxLab). Normalized OIs for the calibration panel were obtained by dividing the test sample OI by the 007sp OI obtained on the same plate, and then multiplying the resultant ratio by the 007sp consensus OI. To estimate the non-normalized and the normalized consensus OIs for the calibration samples, the log transformed OIs and the log transformed normalized OIs 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% CIs for 007sp and calibration

samples were obtained by back-transforming the obtained model intercept and its corresponding 95% CI.

For the purpose of estimating the consensus OIs for the calibration samples for future laboratory comparisons, the normalized individual laboratory geometric mean OIs (GMOI) were screened for outliers relative to the corresponding consensus OI. For each sample, an individual laboratory GMOI was defined to be an inter-laboratory outlier if it differed from its corresponding consensus OI by >4-fold. If, for each combination of sample and serotype, more than one laboratory OI was >4-fold from the consensus OI, then only the most extreme difference was identified as the outlier. Inter-laboratory outliers are listed in Table 2 and are indicated in red font in Supplementary Table S1.

For individual calibration samples, the percent reduction in inter-laboratory variability due to normalization was calculated as $100\% \times \left(1 - \frac{\dot{\sigma}_L^2 + \dot{\sigma}_{R(L)}^2}{\ddot{\sigma}_L^2 + \ddot{\sigma}_{R(L)}^2} \right)$, where $\dot{\sigma}_L^2$ and $\dot{\sigma}_{R(L)}^2$ denote the

between laboratory and run within laboratory variance component estimates for the non-normalized OIs, respectively, and $\ddot{\sigma}_L^2$ and $\ddot{\sigma}_{R(L)}^2$ denote the corresponding variance components for the normalized OIs. In cases where the total variability for the normalized OIs exceeded that for the non-normalized OIs, the percent reduction in inter-laboratory variability

due to normalization was calculated as $100\% \times \left(\frac{\dot{\sigma}_L^2 + \dot{\sigma}_{R(L)}^2}{\ddot{\sigma}_L^2 + \ddot{\sigma}_{R(L)}^2} - 1 \right)$.

To estimate the percent reduction in inter-laboratory variability due to normalization across the set of calibration samples, the log transformed OIs and normalized OIs were fit using a mixed effects ANOVA model containing terms for Lab, Sample, SamplexLab, Run(Lab), SamplexRun(Lab). Sample was regarded as a fixed effect and all other terms as random effects. The percent reduction in inter-laboratory variability due to normalization was calculated

as $100\% \times \left(1 - \frac{\ddot{\sigma}_L^2 + \ddot{\sigma}_{S \times L}^2 + \ddot{\sigma}_{R(L)}^2 + \ddot{\sigma}_{S \times R(L)}^2}{\dot{\sigma}_L^2 + \dot{\sigma}_{S \times L}^2 + \dot{\sigma}_{R(L)}^2 + \dot{\sigma}_{S \times R(L)}^2} \right)$, where $\dot{\sigma}_L^2$, $\dot{\sigma}_{S \times L}^2$, $\dot{\sigma}_{R(L)}^2$ and $\dot{\sigma}_{S \times R(L)}^2$ denote the between

laboratory, sample by laboratory, run within laboratory, and sample by run within laboratory variance component estimates for the non-normalized OIs, respectively, and $\ddot{\sigma}_L^2$, $\ddot{\sigma}_{S \times L}^2$, $\ddot{\sigma}_{R(L)}^2$ and $\ddot{\sigma}_{S \times R(L)}^2$ denote the corresponding variance components for the normalized OIs.

RESULTS

007sp consensus values

For each of the 13 target serotypes, the individual laboratory geometric mean OIs (GMOIs) as well as the consensus value for 007sp are shown in Table 3 and Figure 1. The consensus values ranged from 229 (for serotype 3) to 7776 (serotype 7F). The width of the 95% confidence intervals (CIs) ranged from 1.9-fold for serotype 6A to >10-fold for serotype 19A. The confidence intervals for the other serotypes were generally in the 2- to 3-fold range except for serotype 18C and 23F which had confidence intervals of 5.2- and 4.8-fold, respectively.

The total coefficient of variation (CV, expressed as a percentage) and various variance components (Lab, Run(Lab), and Plate (RunxLab)) estimated from the ANOVA are also shown in Table 3. Serotype 5 had the lowest CV (48%), and serotype 19A had the highest CV (163%). Serotypes 18C and 23F also had relatively high total CVs (127% and 117%, respectively). The total CVs of the remaining serotypes were generally in the 60-80% range. The variance components analysis showed that the laboratory ("Lab" in Table 3) was the major source of variation for all serotypes, except serotype 6A where the variation was mostly associated with the Plate (RunxLab) variance component.

FDA OPA calibration sera normalized consensus values

For each of the FDA OPA calibration sera, OIs were normalized based on the overall 007sp consensus value and the value of 007sp estimated in each corresponding run. The normalized consensus values (and the corresponding 95% CI) for each of the FDA OPA calibration sera were calculated and are shown in Table 4 for the 13 target serotypes. These consensus values were determined after removing outliers (20 outliers were identified amongst the calibration sera, see Table 2). The laboratory specific GMOIs (unadjusted and normalized) for each of the calibration sera are included in Supplementary Table S1 with the 20 results identified as outliers indicated in red. Generally, the confidence intervals for the normalized consensus values are quite small, with most less than 2-fold.

Effect of normalization on inter-laboratory variation

To determine the effect of normalization on assay variation, the variability of the unadjusted results (including inter-laboratory outliers) from the 20 test sera was compared to the variability of the normalized results (also including outliers). For each of the 13 target serotypes, the variability (expressed as %CV) of the unadjusted and normalized results is presented in Table 5. The percent reduction in variability due to normalization, as well as various variance components estimated by the ANOVA, are also indicated in Table 5 (see Supplementary Table S2 for the effect of normalization on individual samples). A noticeable improvement in inter-laboratory agreement with normalization based on 007sp performance was seen, ranging from a 15% reduction in variability for serotype 9V to 64% for serotype 7F. Although the CVs were still high for some serotypes after normalization (for example, for serotype 23F, the CV decreased from 244% to 180% with normalization), the four mock low OI sera are responsible for the high CVs in many instances.

As a visual depiction of the effect of normalization, unadjusted and normalized results are presented graphically in Figure 2. For the indicated serotype, each panel shows the laboratory specific GMOIs (y axis, see legend) as a function of the overall consensus OI (x axis) for each of the 20 sera tested. The left panels display the unadjusted results and the right panels display the normalized results. The results for each sample are connected by a solid black line for clarity. The effect of normalization can be seen by comparing the length of the vertical lines

connecting the data. For most of the sera, normalization resulted in shorter lines, indicating better agreement among the laboratories.

The data in Figure 2 also indicate that the benefit of normalization may be dependent on the OI. For the sera with low consensus values, normalization provided little benefit. In fact, the variability increased for the low OI sera in several instances.

The effect of normalization can also be seen by comparing the breadth of the confidence intervals for the calibration sera in Supplementary Table S2, where normalization reduced the confidence interval for most samples and most serotypes. For some serotypes (19A, for example), the effect is quite striking.

The effect of normalization for each serotype was also assessed by determining the percentage of laboratory specific GMOs (with and without normalization) within 2- and 3-fold of the consensus OI for the 16 FDA OPA calibration sera samples. The resulting percentages combined over the set of laboratories are provided in Table 6. Table 6 also includes the fold range (above and below the overall consensus OIs) needed to encompass 90% of the results was calculated. Normalization resulted in consistently higher percentages of results within 2- and 3-fold of the consensus values, with the increase in results within 2-fold most pronounced. For example, for serotype 19A, the percentage of estimates within 2-fold increases from 56% to 91%.

A similar assessment was also performed for each individual laboratory. The percentages for the individual laboratory assessments are presented in Table 7. For each serotype, 80% of the results were within 2-fold of the consensus values with the following exceptions: Lab B for serotype 19F; Lab E for serotypes 3, 18C, 19F, and 23F; and Lab F for serotype 23F.

Effect of normalization on intra-laboratory variation

As a secondary goal of this study, the impact of standardization on intra-laboratory variation was also examined. The calibration sera results from each run were normalized using the 007sp results from the corresponding run and the 007sp consensus OI. For each combination of laboratory and serotype, the overall intra-laboratory %CV was calculated, with and without normalization, and the reduction in variability due to normalization was determined (see Supplemental Table S3). The results varied considerably, ranging from a 71% reduction in variation (Lab D, serotype 9V) to a 69% increase in variation (Lab E, serotype 9V), with no obvious trend for a particular laboratory nor a particular serotype.

DISCUSSION

In this study, we describe the results of an international collaborative study with two primary goals: 1) assign consensus values for a pneumococcal OPA standard and the FDA OPA calibration sera; and 2) determine the impact of normalization with the OPA standard.

Although different assay formats and lots of reagents were used in this study, we were able to assign the consensus values for 007sp with relatively narrow confidence intervals, suggesting that pneumococcal OPAs produce consistent results despite variations in assay formats and materials among laboratories. For 007sp, there was more variability associated with the consensus values for 18C, 19A, and 23F. For serotypes 18C and 19A, the increased variability was mostly driven by one laboratory (Laboratory E for 18C and Laboratory F for 19A, see Table 3 and Figure 1). The source of the higher variability for serotype 23F is not clear.

Generally, the confidence intervals for the unadjusted results of the FDA OPA calibration sera were comparable to those of 007sp. However, normalization substantively reduced the size of the confidence intervals, with most being less than 2-fold. Both model-based and frequency-based approaches suggested a considerable benefit to normalization in regards to inter-laboratory agreement. The results of the ANOVA indicate at least a 15% reduction in variability due to normalization with 007sp. For the frequency-based approach, the percentage of results within 2- and 3-fold of the consensus values for the 16 calibration sera increases substantially (especially within 2-fold of the consensus values) after normalization (see Table 6).

However, the data in Figure 2 indicate that the benefit of normalization may be dependent on the magnitude of the OI. Normalization did not improve the agreement for samples with low OIs as some labs were not able to detect OIs for these samples. Since undetectable values cannot be normalized, normalization provides little benefit for these low OI sera. Moreover, the confidence intervals of unadjusted estimates for these low titer sera were higher than the calibration sera. The fact that the sera with low OIs are not naturally incurred (i.e., they were created by spiking high OI sera into a negative matrix) may contribute to the increase in variability. Also, the high degree of variability in these sera may be due to the differences in the sensitivity of assays used by different laboratories.

Applying the frequency-based method to the unadjusted data, the percentage of results within 2-fold of the consensus values varied by serotype, ranging from 56% (serotypes 19A and 23F) to 91% (serotype 14), see Table 6. Although differences among individual serotypes were seen, the average of the 13 serotypes (~73%) is quite consistent with that of a previously published study examining the inter-laboratory agreement of OPA data (13) which found that approximately 68% of the results were within 2-fold of consensus values. Based on the results of the previously published study and this study, approximately 70% of the results can be expected to be within 2-fold of the consensus value when unadjusted OPA results from different laboratories are compared, especially when multiple assay formats and/or protocols are utilized. However, our data suggest that normalization can improve overall agreement with more than 90% (average across all serotypes) of the results within 2-fold of the consensus value. In the previous study (13), a 4-fold range above and below (16-fold overall) the consensus value was needed to encompass 90% of the results.

As noted in the method section, normalization of sample data was based on the performance of 007sp on the same assay plate. In order to maximize assay throughput and minimize consumption of 007sp, two alternate analytical approaches were also evaluated: normalizing the data using the average 007sp result from all plates in a run; and normalizing the data using the 007sp result from the first plate of a run. Normalization using either the 007sp OI from the same assay plate or the 007sp OI run average performed similarly in terms of reducing the variation between laboratories (data not shown). However, normalization based on 007sp performance on only the first assay plate was less effective in reducing the variation (data not shown). Therefore, ideally, 007sp would be included 2-3 times in each run.

As a secondary objective of the study, the effect of normalization on intra-laboratory variation was examined. As shown in Supplementary Table S3, the effect of normalization ranged from a 71% decrease in variability to a 69% increase in variability. Although there was no obvious advantage to this approach, the data for this study was collected over a relatively short time frame. Normalization may be useful for improving long-term assay stability when implemented properly. However, normalization should be used cautiously when qualifying new reagents or operators to ensure that it does not mask potential shifts in assay performance.

We see two primary limitations of this study. First, although each participating laboratory was quite experienced with OPAs, the degree and depth of assay validation varied amongst the participating laboratories. Simply using 007sp as a reference standard does not guarantee that every laboratory will generate comparable results. Second, the range of OIs of the calibration sera was limited for some serotypes, and generally lacked low OIs.

Based on the data generated in this study we believe that 007sp may be a useful reference to normalize data across laboratories. We found the most efficient method for normalization to be the use of the 007sp consensus value and the average of the within run estimates using the formula:

$$\text{Normalized OI} = \text{Unadjusted OI} \times \frac{\text{007sp consensus OI (Table 3)}}{\text{007sp OI of run (geometric mean, } n = 2 \text{ or } 3)}$$

The data also support the use of the 16 FDA OPA calibrations sera as part of the initial evaluation of new assays or periodic assessment of established assays. The level of agreement between the laboratory values and the consensus values reported here may help a laboratory evaluate the performance of its assays.

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The University of Alabama at Birmingham (UAB) has IP rights to several reagents developed in M.H.N.'s laboratory, and M.H.N. and R.L.B. are UAB employees. D.W. is an employee of the GSK group of companies. D.C. is an employee of Pfizer Vaccine Research. G.L.X. is an employee of Lanzhou Institute of Biological Products, China.

The authors declare no other conflicts of interest.

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TABLES

Table 1. Participating laboratories and assay formats. The affiliation and location of each participating laboratory is indicated. The assay format (including reference) utilized is also indicated. Note, laboratories are listed alphabetically and this order is not associated with the letter designations (A-F) utilized throughout this manuscript.

Institution Name	Location	OPA Format	Assay Reference
Ewha Womans University	Seoul, Korea	MOPA	(12)
GSK Vaccines	Rixensart, Belgium	Singleplex	(11)
Lanzhou Institute of Biological Products	Lanzhou, China	MOPA	(12)
Pfizer Vaccine Research	Pearl River, New York, USA	Singleplex	(17)
UCL Institute of Child Health	London, England	MOPA	(12)
University of Alabama at Birmingham	Birmingham, Alabama, USA	MOPA	(12)

Table 2. Data excluded from analyses. For each serotype, the calibration sera results identified as intra-laboratory outliers are indicated in the left column, with an intra-laboratory outlier defined as an OI within a laboratory differing by more than 4-fold from the corresponding laboratory's median OI. The right column indicates the number of results identified as inter-laboratory outliers, with an inter-laboratory outlier defined as an laboratory's geometric mean OI differing by more than 4-fold from the corresponding consensus OI.

Intra-Laboratory Outliers		
Serotype	Calibration Serum	Source (# of results)
Pn 1	007E	Laboratory A (1)
	007M	Laboratory D (1)
	007N	Laboratory A (1)
Pn 3	007B	Laboratory F (1)
Pn 4	007E	Laboratory A (1)
	007M	Laboratory A (2)
Pn 6B	007L	Laboratory E (1)
Pn 7F	007M	Laboratory A (2)
Pn 18C	007I	Laboratory E (1)
	007M	Laboratory E (1)
Pn 19A	007I	Laboratory A (1)
Pn 23F	007A	Laboratory D (1)
	007C	Laboratory F (1)
	007F	Laboratory F (2)
	007I	Laboratory A (1)
	007O	Laboratory A (1)
	007O	Laboratory D (1)

Pn, pneumococcal serotype

Inter-Laboratory Outliers		
Serotype	Calibration Serum	Source (# of outliers)
Pn 1	007E	Laboratory A (1)
	007M	Laboratory E (1)
Pn 3	007A	Laboratory E (1)
	007B	Laboratory E (1)
	007E	Laboratory F (1)
	007J	Laboratory E (1)
Pn 6A	007L	Laboratory E (1)
Pn 6B	007L	Laboratory E (1)
Pn 18C	007I	Laboratory E (1)
	007M	Laboratory E (1)
Pn 19A	007E	Laboratory F (1)
	007F	Laboratory F (1)
Pn 19F	007F	Laboratory E (1)
	007L	Laboratory E (1)
Pn 23F	007A	Laboratory F (1)
	007C	Laboratory F (1)
	007F	Laboratory E (1)
	007I	Laboratory F (1)
	007L	Laboratory E (1)
	007O	Laboratory F (1)

Pn, pneumococcal serotype

Table 3. 007sp consensus values and statistics. For each serotype, the laboratory-specific 007sp GMOI and the consensus 007sp OI are shown. Estimates of various variance components and total CV determined by the ANOVA are also indicated.

Serotype	Laboratory Specific GMOI						Consensus OI (95% CI)	Variance Components			Total %CV
	A	B	C	D	E	F		Lab	Run(Lab)	Plate (RunxLab)	
Pn 1	390	700	411	949	1468	556	672 (396, 1143)	0.2496	0.0003	0.0622	75%
Pn 3	187	445	193	494	180	143	229 (131, 398)	0.2710	0.0013	0.0408	75%
Pn 4	2259	9738	2899	4204	3375	3381	3912 (2351, 6511)	0.2264	0.0006	0.0721	73%
Pn 5	688	1181	757	1080	548	519	774 (535, 1119)	0.1180	0.0006	0.0372	48%
Pn 6A	2522	3191	1443	2331	1811	2825	2293 (1683, 3123)	0.0782	0.0001	0.1205	56%
Pn 6B	2893	7668	3915	4515	2849	3430	3976 (2707, 5841)	0.1280	0.0001	0.0804	58%
Pn 7F	7402	15789	4991	9552	12246	3182	7776 (4207, 14371)	0.3360	0.0004	0.0572	87%
Pn 9V	5989	4356	5095	3551	9149	4451	4733 (3083, 7268)	0.1507	0.0031	0.0802	62%
Pn 14	5130	11345	7618	10441	4722	2965	6349 (3680, 10951)	0.2647	0.0000	0.0743	79%
Pn 18C	2518	4475	1618	3806	527	3559	2264 (996, 5145)	0.5996	0.0014	0.0693	127%
Pn 19A	2271	6909	4755	5192	NT	852	3059 (948, 9867)	0.8827	0.0025	0.0463	163%
Pn 19F	2357	1768	1258	3362	869	2055	1766 (1093, 2855)	0.2032	0.0007	0.0369	63%
Pn 23F	1552	3355	1244	1627	6757	820	1952 (895, 4259)	0.5370	0.0036	0.0611	117%

Pn, pneumococcal serotype; **OI**, opsonic index; **GMOI**, geometric mean opsonic index; **CI**, confidence interval; **CV**, coefficient of variation (expressed as a percent); **NT**, not tested; **Lab**, variability among the laboratories; **Run(Lab)**, variability among runs within each laboratory; **Plate(RunxLab)**, variability among plates within each combination of run and laboratory.

Table 4. Normalized consensus OIs for the calibration sera. For each of the calibration sera, the normalized consensus OIs (and 95% CIs) are shown for the indicated serotypes. Outliers were excluded from these analyses.

		Pn 1	Pn 3	Pn 4	Pn 5	Pn 6A	Pn 6B	Pn 7F
007A	Consensus	309	48	5371	475	1270	763	7066
	(95% CI)	(262, 365)	(35, 68)	(4262, 6768)	(338, 670)	(884, 1825)	(500, 1165)	(4403, 11341)
007B	Consensus	264	46	1617	324	1069	831	8157
	(95% CI)	(210, 332)	(22, 95)	(1437, 1821)	(218, 482)	(798, 1433)	(681, 1014)	(5438, 12236)
007C	Consensus	251	29	3890	345	717	2264	8422
	(95% CI)	(216, 292)	(25, 33)	(2582, 5862)	(281, 423)	(519, 991)	(1598, 3207)	(7384, 9606)
007D	Consensus	412	184	10815	1458	20197	13346	22290
	(95% CI)	(248, 684)	(104, 326)	(8833, 13240)	(1085, 1959)	(13192, 30924)	(10226, 17420)	(17233, 28834)
007E	Consensus	114	118	4118	145	2803	3561	9105
	(95% CI)	(30, 428)	(62, 223)	(2755, 6155)	(95, 222)	(2192, 3585)	(2906, 4362)	(6890, 12033)
007F	Consensus	79	35	430	221	1460	1646	2555
	(95% CI)	(54, 114)	(30, 41)	(312, 593)	(165, 296)	(1107, 1924)	(1151, 2354)	(1877, 3478)
007G	Consensus	1777	309	1949	305	2895	6066	10171
	(95% CI)	(1304, 2420)	(255, 375)	(1441, 2637)	(218, 427)	(1793, 4674)	(3204, 11483)	(6628, 15609)
007H	Consensus	128	47	1462	351	1510	1368	2550
	(95% CI)	(86, 190)	(31, 71)	(1272, 1680)	(192, 645)	(1051, 2170)	(961, 1947)	(2123, 3062)
007I	Consensus	118	29	653	93	390	397	3757
	(95% CI)	(90, 157)	(15, 55)	(566, 752)	(63, 136)	(302, 502)	(340, 464)	(2758, 5117)
007J	Consensus	111	7	1205	10	1397	1906	1942
	(95% CI)	(95, 130)	(3, 15)	(995, 1458)	(5, 21)	(1102, 1772)	(1601, 2269)	(1347, 2800)
007K	Consensus	247	39	799	2343	362	610	917
	(95% CI)	(194, 315)	(27, 57)	(584, 1094)	(1320, 4157)	(290, 452)	(490, 761)	(699, 1204)
007L	Consensus	146	54	1459	74	218	317	2570
	(95% CI)	(102, 211)	(46, 64)	(1109, 1921)	(45, 121)	(134, 354)	(237, 422)	(2088, 3163)
007M	Consensus	6	87	2760	155	2413	4186	5450
	(95% CI)	(3, 13)	(60, 127)	(1877, 4059)	(96, 253)	(2128, 2737)	(2921, 5998)	(4137, 7179)
007N	Consensus	157	281	4257	2447	623	1923	6947
	(95% CI)	(111, 220)	(239, 331)	(2990, 6062)	(1801, 3324)	(373, 1041)	(1260, 2937)	(4811, 10030)
007O	Consensus	618	123	8089	123	1172	7438	3947
	(95% CI)	(471, 810)	(84, 180)	(7258, 9015)	(60, 252)	(919, 1495)	(4562, 12127)	(2798, 5567)
007P	Consensus	275	189	3023	525	5171	6526	2384
	(95% CI)	(216, 352)	(132, 270)	(2632, 3471)	(365, 755)	(3888, 6879)	(4930, 8641)	(1862, 3053)

Pn, pneumococcal serotype; OI, opsonic index; CI, confidence interval

Table 4 (continued). Normalized consensus OIs for the calibration sera. For each of the calibration sera, the normalized consensus OIs (and 95% CIs) are shown for the indicated serotypes. Outliers were excluded from these analyses.

		Pn 9V	Pn 14	Pn 18C	Pn 19A	Pn 19F	Pn 23F
007A	Consensus	7771	2289	2218	1252	1910	141
	95% CI	(5943, 10163)	(1680, 3120)	(1522, 3233)	(771, 2032)	(1146, 3181)	(9, 2312)
007B	Consensus	2495	9290	168	693	632	3066
	95% CI	(1653, 3765)	(6915, 12480)	(79, 357)	(433, 1108)	(286, 1396)	(1971, 4768)
007C	Consensus	1010	766	2994	771	613	260
	95% CI	(639, 1595)	(545, 1075)	(2012, 4454)	(616, 964)	(308, 1220)	(187, 360)
007D	Consensus	8591	4339	14662	3519	2671	887
	95% CI	(4927, 14980)	(3590, 5243)	(7071, 30403)	(2063, 6002)	(2034, 3506)	(732, 1076)
007E	Consensus	5067	9051	1646	549	5157	2887
	95% CI	(3835, 6697)	(6450, 12699)	(1069, 2533)	(248, 1215)	(3137, 8479)	(2361, 3531)
007F	Consensus	836	953	451	202	186	124
	95% CI	(639, 1094)	(748, 1214)	(308, 658)	(130, 313)	(113, 307)	(96, 159)
007G	Consensus	2477	7713	1828	1221	2260	6240
	95% CI	(2169, 2830)	(5942, 10014)	(1469, 2276)	(1115, 1336)	(1631, 3133)	(4667, 8343)
007H	Consensus	777	1041	692	638	616	559
	95% CI	(528, 1142)	(737, 1472)	(419, 1143)	(473, 861)	(352, 1077)	(456, 685)
007I	Consensus	829	1567	211	97	604	526
	95% CI	(502, 1371)	(1215, 2022)	(115, 388)	(35, 268)	(339, 1076)	(387, 717)
007J	Consensus	2397	1295	7161	951	950	1086
	95% CI	(1829, 3141)	(960, 1747)	(5433, 9438)	(609, 1485)	(573, 1576)	(900, 1312)
007K	Consensus	1477	530	552	410	230	1051
	95% CI	(947, 2302)	(416, 676)	(292, 1044)	(275, 611)	(127, 417)	(680, 1625)
007L	Consensus	2119	515	325	546	246	168
	95% CI	(1115, 4024)	(375, 708)	(191, 555)	(410, 728)	(188, 322)	(124, 228)
007M	Consensus	1195	5264	1325	1670	1445	957
	95% CI	(958, 1492)	(3594, 7710)	(754, 2327)	(1285, 2170)	(1217, 1716)	(830, 1103)
007N	Consensus	16503	2789	6889	1388	2669	910
	95% CI	(11321, 24055)	(2005, 3879)	(5130, 9251)	(783, 2460)	(1937, 3677)	(652, 1270)
007O	Consensus	1507	5731	2022	2171	522	383
	95% CI	(744, 3056)	(4247, 7732)	(1596, 2562)	(1161, 4058)	(283, 961)	(177, 825)
007P	Consensus	2154	3656	732	1525	902	1627
	95% CI	(1483, 3128)	(2814, 4751)	(538, 995)	(1147, 2026)	(670, 1215)	(1398, 1894)

Pn, pneumococcal serotype; OI, opsonic index; CI, confidence interval

Table 5. Model-based assessment of the effect of normalization. The overall reduction in variability due to normalization is shown for each serotype. Estimates of various variance components and CVs of the unadjusted and normalized results from the ANOVA are also shown.

	Unadjusted					Normalized					Variability Reduction (%)
	Variance Components					Variance Components					
	Lab	LabxSample	Run(Lab)	Samplex Run(Lab)	%CV	Lab	LabxSample	Run(Lab)	Samplex Run(Lab)	%CV	
Pn1	0.2456	0.3424	0.0103	0.0631	126%	0.0000	0.3099	0.0196	0.0646	87%	40%
Pn3	0.2848	0.2032	0.0197	0.0687	114%	0.0457	0.1729	0.0180	0.0735	75%	46%
Pn4	0.3046	0.3975	0.0218	0.0632	143%	0.0290	0.4374	0.0135	0.0696	110%	30%
Pn5	0.1437	0.1511	0.0096	0.0480	81%	0.0200	0.1236	0.0135	0.0572	59%	39%
Pn6A	0.1088	0.2606	0.0304	0.0900	101%	0.0185	0.2810	0.0096	0.0941	89%	18%
Pn6B	0.2177	0.3247	0.0244	0.0578	120%	0.0251	0.3774	0.0290	0.0657	102%	20%
Pn7F	0.6062	0.0750	0.0208	0.0781	142%	0.0574	0.0897	0.0198	0.1113	69%	64%
Pn9V	0.0702	0.3027	0.0501	0.0771	103%	0.0310	0.2594	0.0315	0.1010	92%	15%
Pn14	0.1515	0.0451	0.0286	0.0465	68%	0.0275	0.0427	0.0073	0.0491	43%	53%
Pn18C	0.9021	0.3757	0.0398	0.1014	229%	0.1726	0.5400	0.0232	0.0999	149%	41%
Pn19A	1.1479	0.1970	0.0282	0.0501	230%	0.1144	0.3457	0.0113	0.0940	112%	60%
Pn19F	0.4291	0.1988	0.0297	0.0681	134%	0.1736	0.1790	0.0221	0.0840	97%	37%
Pn23F	0.4649	0.9584	0.0299	0.0739	244%	0.0673	0.9215	0.0072	0.0666	180%	30%

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

Table 6. Frequency-based assessment of the effect of normalization. The percentage of laboratory-specific OIs within 2- and 3-fold of the overall consensus value is shown below. The fold range from the overall consensus needed to include 90% of the values ("Fold (90%)") is also shown. Outlier results are included in this analysis.

	Unadjusted			Normalized		
	2-fold	3-fold	Fold (90%)	2-fold	3-fold	Fold (90%)
Pn 1	73%	92%	2.90	91%	96%	1.95
Pn 3	60%	90%	3.00	88%	96%	2.13
Pn 4	79%	96%	2.49	99%	100%	1.49
Pn 5	73%	97%	2.40	92%	100%	1.96
Pn 6A	89%	98%	2.04	94%	99%	1.76
Pn 6B	83%	98%	2.25	96%	99%	1.70
Pn 7F	67%	81%	3.53	99%	100%	1.60
Pn 9V	86%	100%	2.10	94%	99%	1.90
Pn 14	91%	99%	1.95	100%	100%	1.49
Pn 18C	63%	77%	5.30	80%	97%	2.18
Pn 19A	56%	71%	4.50	91%	95%	1.80
Pn 19F	75%	90%	3.00	82%	97%	2.23
Pn 23F	56%	73%	5.11	75%	87%	3.20

Pn, pneumococcal serotype

Table 7. Frequency-based assessment of normalization for individual laboratories. For each laboratory, the percentages of results within 2- and 3-fold of the consensus values listed in Table 4 are indicated. Outliers were

included in these analyses. Results less than 80% for 2-fold are indicated by gray shading.

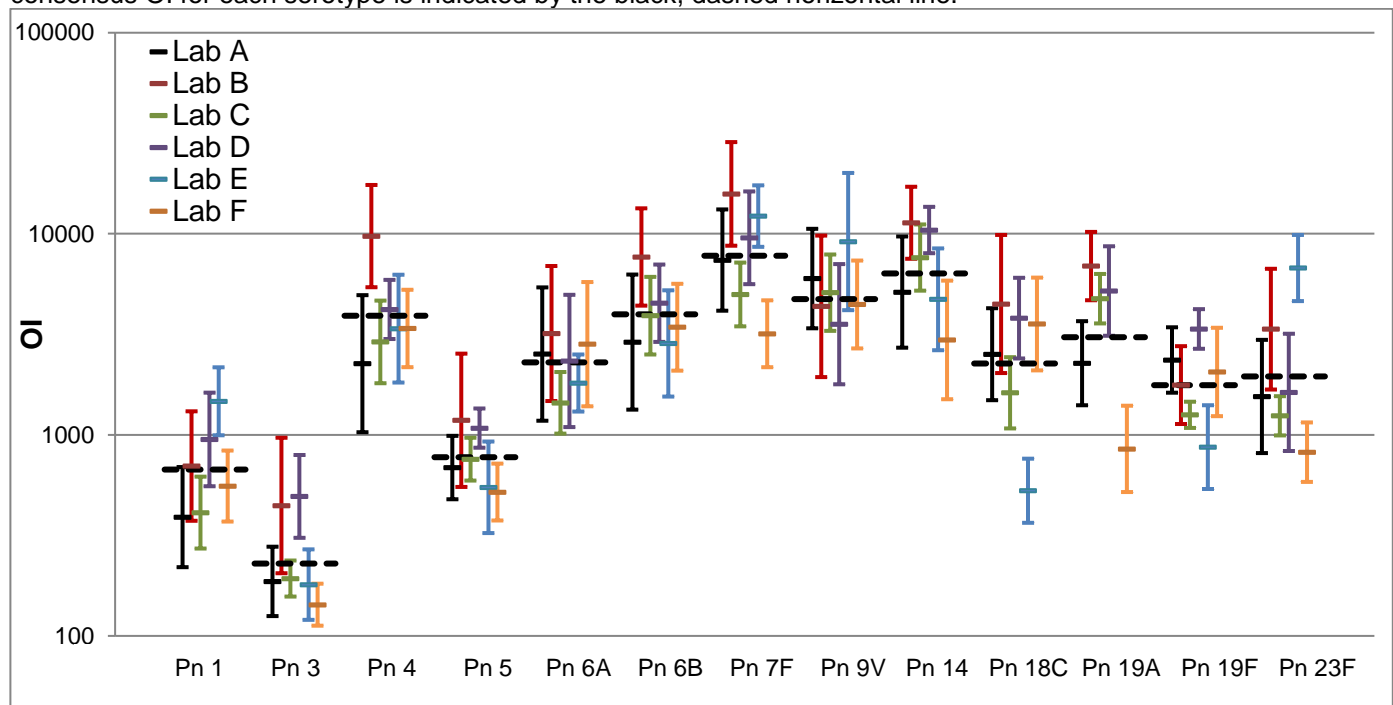
Lab	Pn1		Pn3		Pn4		Pn5		Pn6A		Pn6B		Pn7F	
	2-fold	3-fold	2-fold	3-fold	2-fold	3-fold	2-fold	3-fold	2-fold	3-fold	2-fold	3-fold	2-fold	3-fold
A	94%	94%	100%	100%	100%	100%	81%	100%	100%	100%	100%	100%	100%	100%
B	94%	94%	94%	100%	100%	100%	94%	100%	100%	100%	100%	100%	94%	100%
C	94%	100%	100%	100%	100%	100%	94%	100%	100%	100%	100%	100%	100%	100%
D	94%	94%	94%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
E	88%	94%	63%	81%	100%	100%	81%	100%	88%	94%	81%	94%	100%	100%
F	94%	94%	88%	94%	100%	100%	94%	100%	94%	100%	100%	100%	100%	100%

	Pn9V		Pn14		Pn18C		Pn19A		Pn19F		Pn23F	
	2-fold	3-fold	2-fold	3-fold	2-fold	3-fold	2-fold	3-fold	2-fold	3-fold	2-fold	3-fold
A	100%	100%	100%	100%	94%	100%	100%	100%	94%	100%	94%	100%
B	94%	100%	100%	100%	100%	100%	81%	100%	63%	100%	100%	100%
C	94%	100%	100%	100%	94%	100%	100%	100%	100%	100%	100%	100%
D	100%	100%	100%	100%	81%	100%	100%	100%	94%	100%	88%	94%
E	88%	94%	100%	100%	69%	88%	NT	NT	50%	81%	75%	81%
F	100%	100%	100%	100%	100%	100%	81%	81%	100%	100%	75%	75%

Pn, pneumococcal serotype

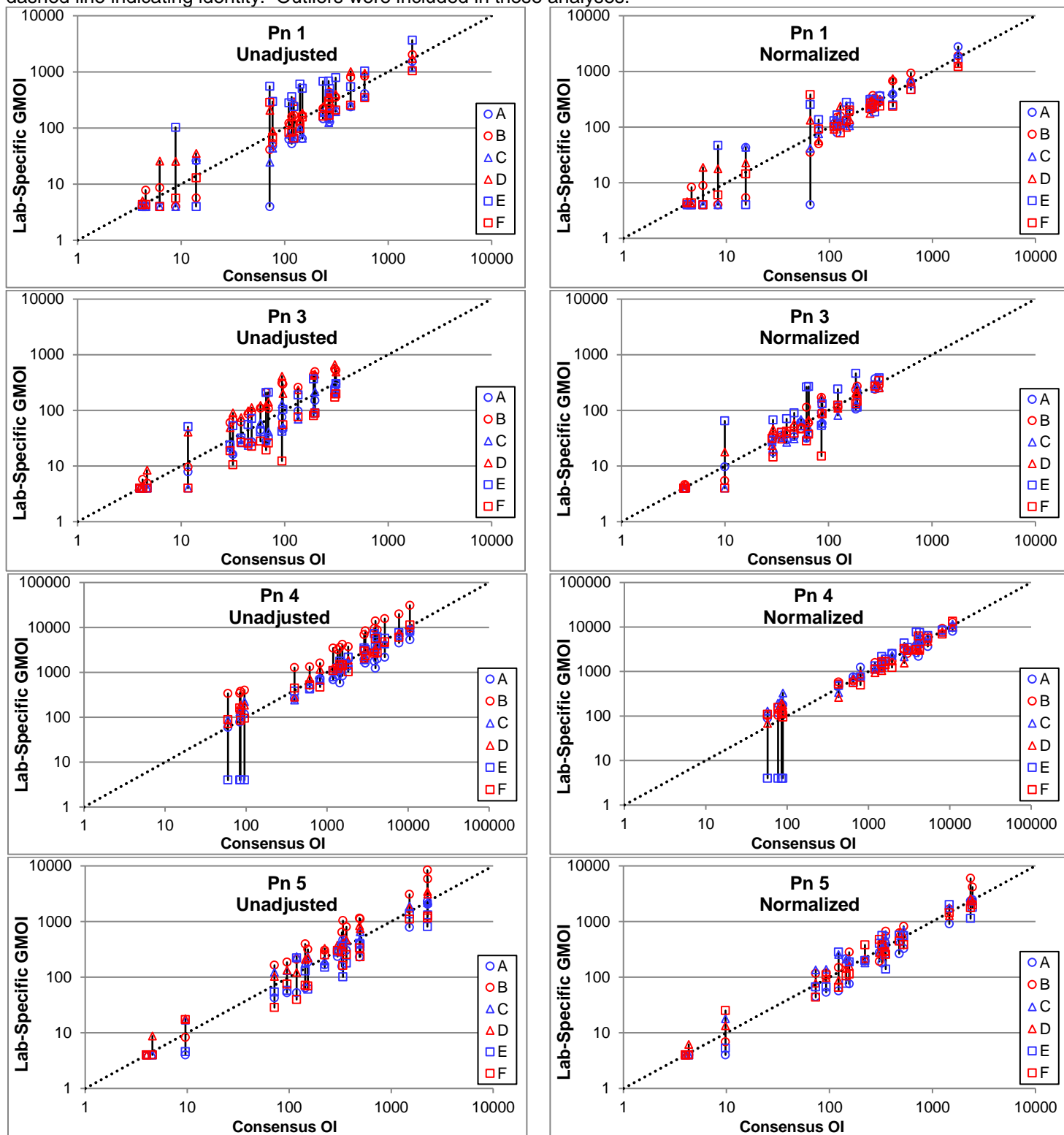
FIGURES

Figure 1. 007sp results. For each serotype (x-axis), the line representing each laboratory (see legend) indicates the laboratory specific GMOI (y-axis, center tick) and +/- 2 standard deviations (y-axis, terminal ticks). The consensus OI for each serotype is indicated by the black, dashed horizontal line.



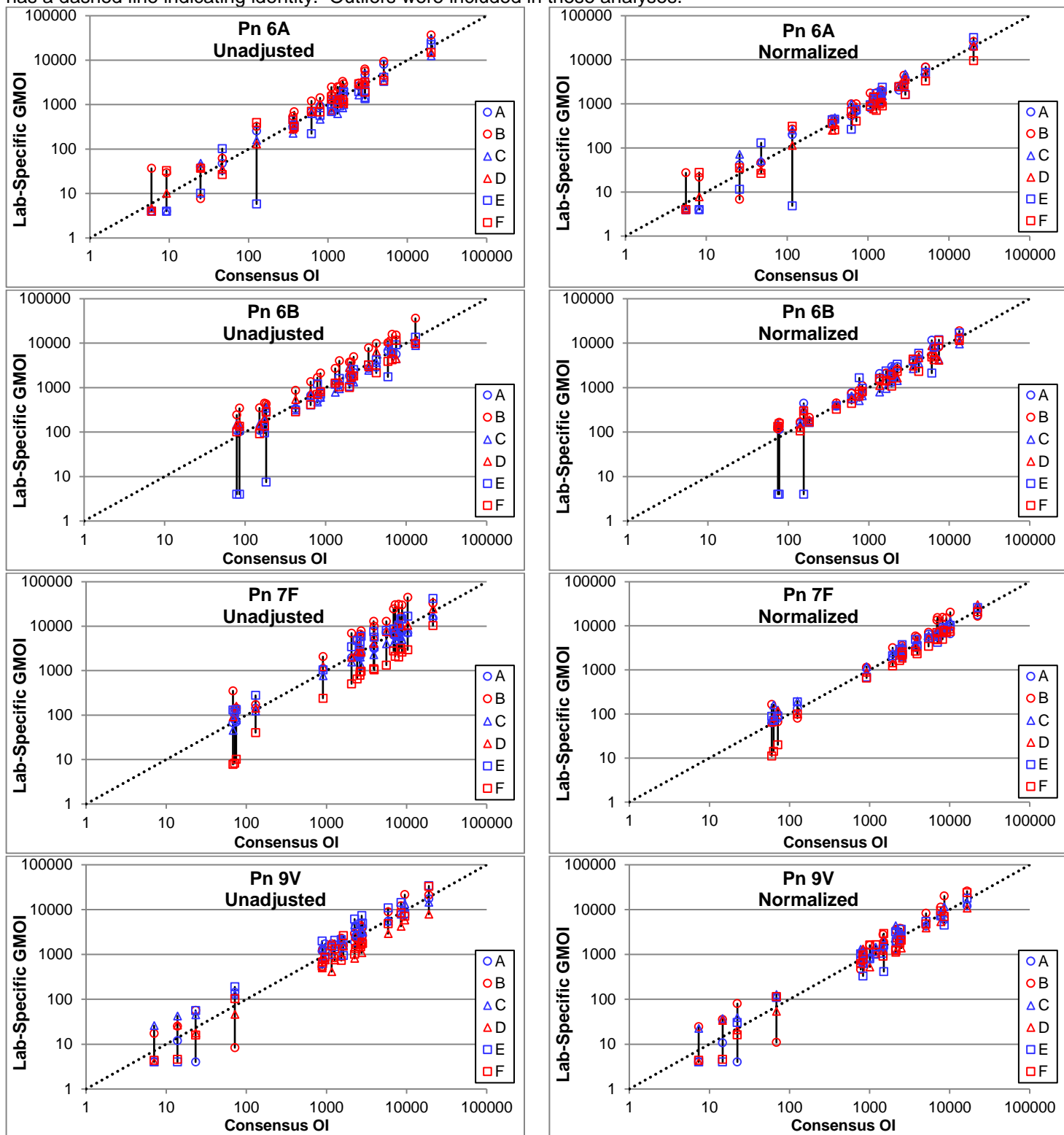
Pn, pneumococcal serotype; OI, opsonic index; GMOI, geometric mean opsonic index

Figure 2. Results of normalization. For the 20 sera tested, each plot shows the six laboratory-specific GMOIs (y axis) as a function of the consensus OI (x axis). Unadjusted data is displayed in the left plots, and normalized data on the right plots. The laboratory-specific GMOIs for each sample are connected by a vertical line for visualization. Each plot also has a dashed line indicating identity. Outliers were included in these analyses.



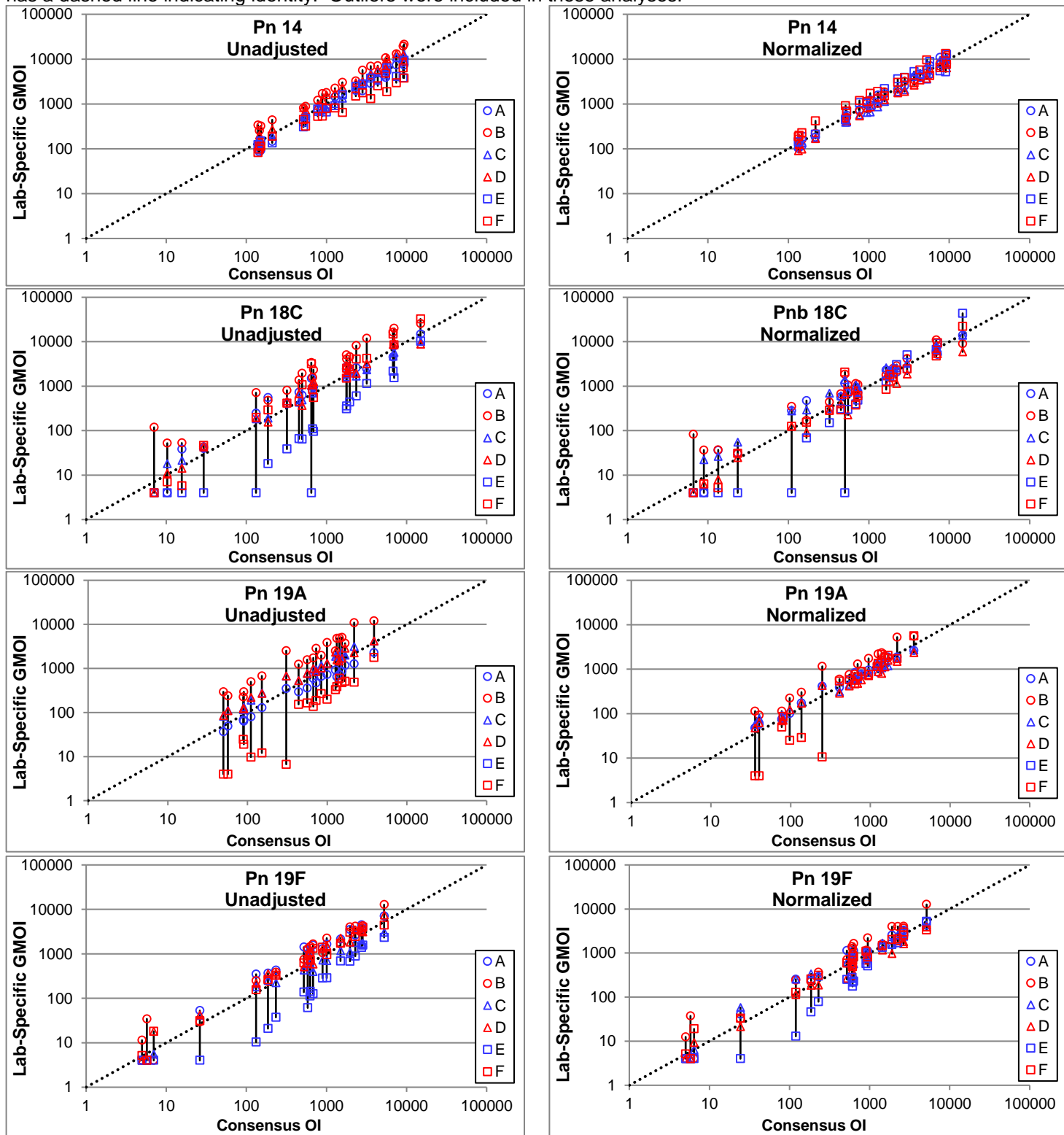
Pn, Pneumococcal serotype; OI, opsonic index; GMOI, geometric mean opsonic index

Figure 2 (continued). Results of normalization. For the 20 sera tested, each plot shows the six laboratory-specific OIs (y axis) as a function of the consensus OI (x axis). Unadjusted data is displayed in the left plots, and normalized data on the right plots. The laboratory-specific OIs for each sample are connected by a vertical line for visualization. Each plot also has a dashed line indicating identity. Outliers were included in these analyses.



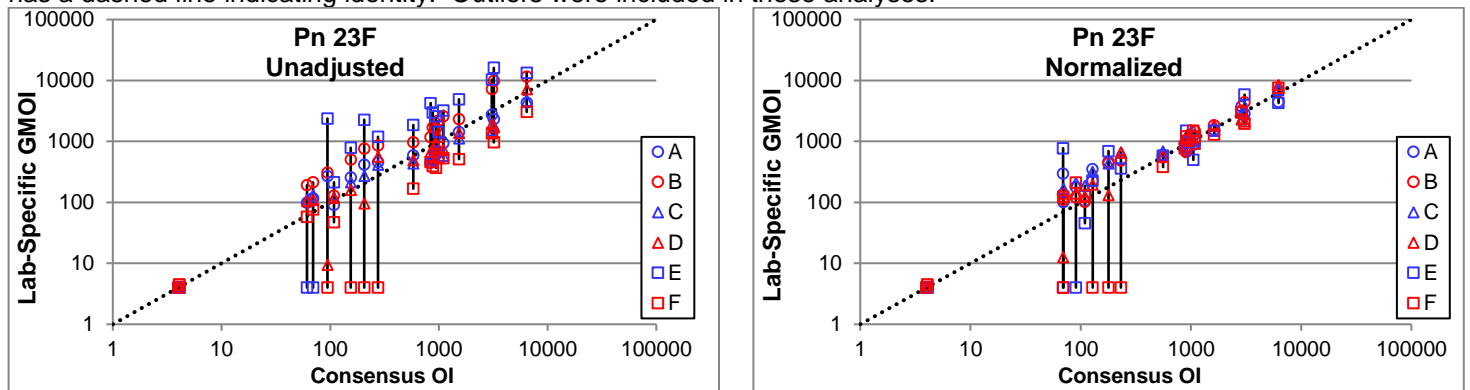
Pn, Pneumococcal serotype; OI, opsonic index; GMOI, geometric mean opsonic index

Figure 2 (continued). Results of normalization. For the 20 sera tested, each plot shows the six laboratory-specific OIs (y axis) as a function of the consensus OI (x axis). Unadjusted data is displayed in the left plots, and normalized data on the right plots. The laboratory-specific OIs for each sample are connected by a vertical line for visualization. Each plot also has a dashed line indicating identity. Outliers were included in these analyses.



Pn, Pneumococcal serotype; OI, opsonic index; GMOI, geometric mean opsonic index

Figure 2 (continued). Results of normalization. For the 20 sera tested, each plot shows the six laboratory-specific OIs (y axis) as a function of the consensus OI (x axis). Unadjusted data is displayed in the left plots, and normalized data on the right plots. The laboratory-specific OIs for each sample are connected by a vertical line for visualization. Each plot also has a dashed line indicating identity. Outliers were included in these analyses.



Pn, Pneumococcal serotype; **OI**, opsonic index; **GMOI**, geometric mean opsonic index

SUPPLEMENTAL MATERIALS

Supplementary Table S1. Test sera OIs. The consensus and the laboratory-specific GMOIs for the unadjusted and normalized results are shown for each serum tested in this study. Results identified as outliers are indicated in red. Outliers were included in the determination of the consensus values in this table, but are excluded in other indicated analyses.

Serotype	Sample	Unadjusted							Normalized						
		Consensus OI	Laboratory-Specific GMOI						Consensus OI	Laboratory-Specific GMOI					
			Lab A	Lab B	Lab C	Lab D	Lab E	Lab F		Lab A	Lab B	Lab C	Lab D	Lab E	Lab F
Pn 1	FDA007A	311	199	353	195	400	789	209	309	366	301	315	291	361	238
	FDA007B	272	128	431	150	446	503	221	264	212	366	234	325	230	251
	FDA007C	266	142	265	125	366	688	300	251	231	225	214	267	315	270
	FDA007D	434	240	799	246	1020	546	255	412	389	680	424	743	250	235
	FDA007E	72	4	41	25	208	556	285	65	4	35	42	133	254	384
	FDA007F	77	48	54	44	88	297	68	79	87	50	76	56	136	92
	FDA007G	1709	1532	2031	1205	1735	3668	1044	1777	2786	1903	2079	1387	1680	1225
	FDA007H	123	65	169	64	298	242	66	128	119	158	111	238	111	79
	FDA007I	117	52	160	64	158	359	85	118	79	149	109	126	165	103
	FDA007J	110	63	122	78	126	281	83	111	103	114	134	92	129	101
	FDA007K	234	147	224	189	241	679	163	247	241	250	325	176	311	214
	FDA007L	141	79	123	99	135	607	99	146	128	137	158	98	278	130
	FDA007M	9	4	4	4	26	103	6	8	4	4	4	18	47	6
	FDA007N	148	65	168	65	186	508	157	157	115	187	105	135	233	202
	FDA007O	592	409	836	369	948	1032	347	618	653	934	595	689	473	470
	FDA007P	270	188	347	187	454	408	170	275	300	332	309	329	187	230
	S21	4	4	4	4	5	4	4	4	4	4	4	5	4	4
S22	5	4	8	4	4	4	4	5	4	8	4	4	4	4	
S23	14	26	6	27	35	4	13	15	43	5	43	23	4	14	
S24	6	4	9	4	26	4	4	6	4	9	4	19	4	4	
Pn 3	FDA007A	69	33	109	41	141	212	26	64	37	59	48	67	269	37
	FDA007B	66	28	208	29	124	208	19	61	31	113	34	59	264	28
	FDA007C	30	24	60	22	49	24	19	29	29	33	27	23	29	31
	FDA007D	190	87	428	102	427	364	81	184	105	233	127	204	463	131
	FDA007E	94	76	312	130	412	42	12	86	91	170	162	168	53	15
	FDA007F	38	29	62	26	75	32	27	35	36	33	33	30	40	40
	FDA007G	313	298	523	206	490	302	197	309	368	279	258	253	384	340
	FDA007H	48	27	91	27	113	71	23	47	34	49	31	58	90	39
	FDA007I	32	16	69	18	90	53	11	29	18	37	20	46	67	15
	FDA007J	12	8	10	4	41	51	4	10	10	5	4	18	65	4
	FDA007K	44	27	86	24	99	56	26	39	29	37	27	43	71	42
	FDA007L	58	44	109	59	123	41	28	54	61	47	69	53	52	46
	FDA007M	96	46	297	50	201	104	55	87	58	129	58	88	132	86
	FDA007N	305	267	552	212	665	226	172	281	371	239	248	290	287	270
	FDA007O	134	97	258	70	233	190	76	123	118	112	81	109	242	124
	FDA007P	196	150	492	217	447	90	89	189	186	272	264	209	114	145
	S21	4	4	4	4	4	4	4	4	4	4	4	4	4	4
S22	4	4	6	4	4	4	4	4	4	5	4	4	4	4	
S23	5	4	5	4	8	4	4	4	4	4	4	5	4	4	
S24	4	4	4	4	4	4	4	4	4	4	4	4	4	4	

Pn, pneumococcal serotype; OI, opsonic index; GMOI, geometric mean opsonic index

Supplementary Table S1 (continued). Test sera OIs. The consensus and the laboratory-specific OIs for the unadjusted and normalized results are shown for each serum tested in this study. Results identified as outliers are indicated in red. Outliers were included in the determination of the consensus values in this table, but are excluded in other indicated analyses.

Serotype	Sample	Unadjusted							Normalized						
		Consensus OI	Laboratory-Specific GMOI						Consensus OI	Laboratory-Specific GMOI					
			Lab A	Lab B	Lab C	Lab D	Lab E	Lab F		Lab A	Lab B	Lab C	Lab D	Lab E	Lab F
Pn 4	FDA007A	5158	2176	15743	4290	4643	5722	4821	5371	3651	6390	5988	4790	6494	5604
	FDA007B	1542	831	4195	1196	1395	1586	1460	1617	1395	1703	1669	1439	1910	1697
	FDA007C	3849	1766	9503	3415	2913	7286	2671	3890	2639	3857	4767	3005	7754	3124
	FDA007D	10516	5359	31260	8267	9812	8743	11381	10815	8010	12688	11540	10122	9897	13478
	FDA007E	3955	1238	13933	3182	3362	3643	5638	4118	2218	5655	4442	3168	4285	6317
	FDA007F	396	262	1276	242	280	382	447	430	483	583	340	264	488	526
	FDA007G	1822	1385	3748	1654	1881	2180	1039	1949	2552	1712	2328	1741	2535	1230
	FDA007H	1350	855	3186	1089	1317	1261	1229	1462	1575	1455	1533	1219	1606	1445
	FDA007I	610	434	1333	541	729	425	531	653	757	609	762	675	572	562
	FDA007J	1189	684	3467	792	1257	1088	1098	1205	1192	1584	1114	950	1347	1131
	FDA007K	820	719	1611	729	1167	654	471	799	1254	749	828	882	759	499
	FDA007L	1440	580	3584	1151	1404	1769	1500	1459	1180	1665	1307	1061	2189	1673
	FDA007M	2873	1931	6932	1899	2064	3496	3012	2760	2906	3220	2156	1561	4379	3304
	FDA007N	4163	2006	8837	4444	3958	6169	2707	4257	4083	4105	5046	2993	7616	3154
	FDA007O	7735	4519	19939	6759	7665	7651	5995	8088	8585	9261	7675	7736	8573	6995
	FDA007P	2970	1614	8412	2240	2994	3214	2347	3023	3066	2545	3332	3021	3505	2939
	S17	84	79	343	132	150	4	161	77	118	104	197	142	4	157
S18	96	119	401	222	187	4	95	89	178	121	331	177	4	95	
S19	60	59	338	88	73	4	88	58	89	102	131	69	4	110	
S20	85	100	379	157	188	4	85	86	167	115	233	194	4	112	
Pn 5	FDA007A	489	230	1150	459	839	402	335	475	263	625	458	615	566	445
	FDA007B	329	156	639	338	366	358	285	324	178	347	337	268	562	379
	FDA007C	364	304	803	449	469	179	253	345	351	436	415	344	262	288
	FDA007D	1513	785	3087	1891	1739	1370	1101	1458	906	1676	1747	1276	2004	1432
	FDA007E	153	186	317	229	221	61	70	145	215	172	212	146	77	105
	FDA007F	223	165	305	195	328	151	251	221	196	197	180	217	199	380
	FDA007G	298	234	297	314	377	282	302	305	278	191	290	275	402	475
	FDA007H	337	462	1035	457	407	101	163	351	549	667	479	297	140	256
	FDA007I	95	53	185	132	134	58	75	93	53	120	138	98	67	109
	FDA007J	10	4	8	17	18	5	17	10	4	7	18	13	5	25
	FDA007K	2265	2299	8379	2171	3437	811	1159	2343	2335	5974	2280	2586	1130	1771
	FDA007L	72	43	164	123	103	54	28	74	45	117	135	78	66	43
	FDA007M	144	63	394	183	209	129	71	155	76	281	200	157	187	113
	FDA007N	2279	2054	5841	2295	3111	1307	1251	2447	2454	4164	2507	2341	1783	1992
	FDA007O	118	53	208	235	122	222	40	123	57	148	256	87	281	65
	FDA007P	498	311	1112	653	742	391	233	525	327	820	689	531	554	384
	S21	5	4	4	4	9	4	4	4	4	4	4	4	6	4
S22	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
S23	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
S24	4	4	4	4	4	4	4	4	4	4	4	4	4	4	

Pn, pneumococcal serotype; OI, opsonic index; GMOI, geometric mean opsonic index

Supplementary Table S1 (continued). Test sera OIs. The consensus and the laboratory-specific OIs for the unadjusted and normalized results are shown for each serum tested in this study. Results identified as outliers are indicated in red. Outliers were included in the determination of the consensus values in this table, but are excluded in other indicated analyses.

Serotype	Sample	Unadjusted							Normalized						
		Consensus OI	Laboratory-Specific GMOI						Consensus OI	Laboratory-Specific GMOI					
			Lab A	Lab B	Lab C	Lab D	Lab E	Lab F		Lab A	Lab B	Lab C	Lab D	Lab E	Lab F
Pn 6A	FDA007A	1316	1356	2510	624	1775	1284	1073	1270	1268	1776	1045	1474	1711	712
	FDA007B	1104	862	2482	697	1314	698	1327	1069	806	1756	1150	1091	949	881
	FDA007C	795	930	1424	467	1040	571	683	717	674	1007	759	863	742	404
	FDA007D	19973	27515	36739	12627	14864	22365	14964	20197	19943	25993	21260	20427	31910	9603
	FDA007E	2906	3421	6303	1436	2920	2078	3202	2803	2480	4460	2418	2511	2634	2733
	FDA007F	1534	1847	3344	850	1306	1472	1293	1460	1447	2068	1431	1123	1890	1081
	FDA007G	2930	4679	5683	2818	3239	1365	1911	2895	3667	3515	4745	3589	1612	1641
	FDA007H	1576	2239	2956	1000	1049	1916	1154	1510	1755	1829	1516	1162	2370	897
	FDA007I	375	366	693	323	307	344	322	390	433	429	490	340	446	256
	FDA007J	1400	1209	2524	1262	1649	935	1267	1397	1430	1561	1913	1458	1167	1006
	FDA007K	362	307	553	228	284	445	459	362	364	456	354	251	435	359
	FDA007L	126	258	324	159	130	6	398	116	201	268	256	115	5	311
	FDA007M	2444	2651	3042	1648	2793	1947	2949	2413	2064	2509	2597	2471	2418	2459
	FDA007N	619	684	1212	631	689	223	704	623	533	999	1005	609	265	668
	FDA007O	1105	1279	1648	771	724	1034	1498	1172	1088	1359	1229	764	1314	1429
	FDA007P	5047	8001	9370	3325	4586	4160	3477	5171	6803	6920	4890	4840	5175	3318
	S21	6	4	37	4	5	4	4	6	4	27	4	4	4	4
S22	46	48	62	35	36	102	27	48	47	46	51	31	131	26	
S23	25	40	8	48	40	10	37	26	41	7	72	35	12	35	
S24	9	4	30	4	10	4	33	8	4	22	4	8	4	28	
Pn 6B	FDA007A	789	509	1673	470	714	1216	692	763	618	875	509	639	1655	712
	FDA007B	861	603	2103	602	903	737	803	831	732	1100	651	808	963	826
	FDA007C	2225	2196	4933	1328	1822	2540	1823	2264	2953	2580	1437	1631	3347	2289
	FDA007D	13019	10081	36171	8833	11410	13666	9694	13346	13554	18921	9553	11410	16880	12174
	FDA007E	3400	2707	7763	2451	2917	3197	3216	3561	3640	4061	2650	3032	3987	4350
	FDA007F	1479	1172	4022	942	1416	1610	1035	1646	2063	2115	950	1471	2353	1401
	FDA007G	5929	6602	10122	8614	11099	1747	3893	6066	11623	5323	8685	8857	2125	4894
	FDA007H	1313	1169	2706	783	1397	1178	1258	1368	2059	1423	789	1115	1618	1581
	FDA007I	423	333	860	405	539	315	283	397	386	453	408	430	389	326
	FDA007J	2068	1316	3754	2479	2537	1499	1680	1906	1523	1974	2499	1862	1748	1935
	FDA007K	651	663	1360	665	757	405	414	610	767	741	646	555	574	439
	FDA007L	182	274	427	347	421	7	280	155	443	233	337	309	4	297
	FDA007M	4247	2854	9838	3664	6436	4148	2135	4186	4451	5364	3559	4722	5915	2291
	FDA007N	1974	1829	3733	2920	2785	1066	1001	1923	2953	2035	2836	2044	1337	1074
	FDA007O	7497	5634	15083	4446	4498	9108	11473	7438	8474	8224	4318	4120	11972	11467
	FDA007P	6680	5680	15787	5798	6647	6138	4190	6527	8544	7613	5827	6087	8075	4187
	S17	85	102	343	132	153	4	134	77	113	165	132	137	4	153
S18	151	110	349	155	142	IR	92	141	157	168	156	127	IR	105	
S19	174	123	443	178	211	96	137	183	176	214	179	188	163	166	
S20	79	108	243	152	147	4	100	74	132	117	152	131	4	135	

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Supplementary Table S1 (continued). Test sera OIs. The consensus and the laboratory-specific OIs for the unadjusted and normalized results are shown for each serum tested in this study. Results identified as outliers are indicated in red. Outliers were included in the determination of the consensus values in this table, but are excluded in other indicated analyses.

Serotype	Sample	Unadjusted							Normalized						
		Consensus OI	Laboratory-Specific GMOI						Consensus OI	Laboratory-Specific GMOI					
			Lab A	Lab B	Lab C	Lab D	Lab E	Lab F		Lab A	Lab B	Lab C	Lab D	Lab E	Lab F
Pn 7F	FDA007A	7210	6112	30589	5029	10448	6811	2100	7066	6816	15312	7573	7655	4213	4857
	FDA007B	7977	6295	31180	5932	10382	10579	2016	8157	7019	15607	8932	8944	6754	4964
	FDA007C	8464	8224	16203	5873	10248	14468	3170	8422	8716	8111	8748	8109	9195	7873
	FDA007D	21335	15216	33082	17430	24714	42089	10334	22290	17559	16560	25964	30483	25921	20993
	FDA007E	8781	7765	30178	5032	10159	14568	2627	9105	8231	15106	8683	8163	9194	7036
	FDA007F	2698	2427	7838	1331	2627	5955	974	2555	2302	3562	1983	1833	3738	2559
	FDA007G	10317	7011	44663	7411	10535	16766	2943	10171	6648	20297	12513	8441	10313	7534
	FDA007H	2634	2173	6647	2017	2732	4485	936	2550	2060	3021	3103	2189	2758	2380
	FDA007I	3876	3337	12956	2281	4120	7585	1101	3757	3219	5888	3510	3301	4882	2669
	FDA007J	2056	1944	6997	1574	2008	3465	507	1942	1875	3180	2422	1439	2110	1228
	FDA007K	904	1121	2067	769	1237	1038	239	917	1081	1147	1183	886	690	655
	FDA007L	2619	2195	5752	2356	3480	3944	790	2570	2130	3190	3337	2495	2329	2162
	FDA007M	5594	7271	12912	4062	7543	8021	1333	5450	6447	7162	5930	5407	5299	3415
	FDA007N	6884	6285	24348	4124	7028	8649	2775	6947	6098	13506	6743	5038	5639	7046
	FDA007O	3952	3148	9995	3446	6014	5634	1037	3947	3383	5544	5030	4873	3511	2333
	FDA007P	2418	2167	5096	2185	2674	4785	648	2384	2375	2375	3190	2167	2929	1640
	S21	72	78	140	110	117	117	8	63	73	65	161	83	72	14
S22	68	67	353	45	92	131	8	60	63	165	71	65	89	11	
S23	130	143	171	125	144	278	40	126	134	80	196	102	188	99	
S24	75	71	144	78	160	134	10	72	79	67	123	117	89	20	
Pn 9V	FDA007A	8502	7841	12063	7926	4239	14271	8327	7771	6892	11229	7748	5400	7237	9374
	FDA007B	2753	2306	4043	3142	1090	7306	1869	2495	2027	3763	3071	1389	3607	2068
	FDA007C	1161	1454	1714	988	413	1562	1541	1010	1130	1595	860	527	800	1613
	FDA007D	9509	7626	21546	13367	5873	8073	7098	8591	5926	20055	11633	9099	4504	7026
	FDA007E	5874	5515	8860	5528	2899	10896	4815	5067	4286	8247	4811	3872	5413	4762
	FDA007F	966	1729	826	760	757	1154	855	836	1158	717	661	1011	622	974
	FDA007G	2825	3940	2656	3137	1805	4951	1732	2477	2639	2304	2730	2650	2544	2077
	FDA007H	882	1183	533	1377	500	1988	544	777	793	462	1308	734	1021	618
	FDA007I	896	1321	1214	854	897	625	673	829	1085	1053	811	1317	331	781
	FDA007J	2676	2871	4400	2537	1632	4781	1470	2397	2359	3816	2410	2233	2206	1762
	FDA007K	1533	1877	2284	2149	897	2146	731	1477	1543	2784	2042	1226	1053	909
	FDA007L	2246	1902	2899	4611	817	6158	1003	2119	1325	3533	4381	1117	3179	1247
	FDA007M	1268	1637	843	1482	749	2051	1325	1195	1140	1028	1461	1024	1003	1644
	FDA007N	18982	17312	21153	14539	7888	33909	32852	16503	12055	25783	14332	10788	17768	23732
	FDA007O	1621	1880	1561	1642	1533	941	2608	1507	1440	1902	1618	2160	416	2919
	FDA007P	2310	2539	2341	2876	1663	3927	1360	2154	1945	3316	2835	2342	1915	1212
	S21	72	142	8	129	46	189	103	69	114	11	127	54	109	114
S22	7	4	17	26	4	4	4	7	4	24	22	4	4	4	
S23	23	4	57	45	18	56	16	22	4	80	39	21	30	16	
S24	14	12	25	43	27	4	5	15	11	35	37	34	4	5	

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Serotype	Sample	Unadjusted							Normalized						
		Consensus OI	Laboratory-Specific GMOI						Consensus OI	Laboratory-Specific GMOI					
			Lab A	Lab B	Lab C	Lab D	Lab E	Lab F		Lab A	Lab B	Lab C	Lab D	Lab E	Lab F
Pn 14	FDA007A	2326	1998	3252	2209	3021	2445	1495	2289	2146	1863	1874	1771	3625	3025
	FDA007B	9299	7398	21492	12486	9124	9421	3789	9290	7946	12315	10590	6391	12732	7669
	FDA007C	780	729	1214	680	924	762	530	766	738	695	577	541	1070	1191
	FDA007D	4369	3518	7151	5488	5836	3388	2548	4339	3883	4097	4655	3421	4581	5725
	FDA007E	9073	8337	19540	11533	12171	3835	6358	9051	8856	11197	9782	8003	5194	13392
	FDA007F	888	775	1718	765	1233	731	533	953	1161	1052	646	811	1049	1122
	FDA007G	7439	7338	13082	11043	13093	4100	2978	7713	10998	8007	9317	7090	5500	6498
	FDA007H	998	823	1798	789	1603	665	796	1041	1234	1100	665	868	935	1736
	FDA007I	1579	1563	3033	1352	2315	1604	650	1567	1694	1856	1141	1253	2200	1526
	FDA007J	1276	1447	2245	1019	1759	917	810	1295	1568	1374	859	1042	1284	1901
	FDA007K	549	520	886	541	720	476	319	530	564	501	398	427	673	695
	FDA007L	516	382	810	530	834	306	450	515	521	457	389	495	443	917
	FDA007M	5489	3924	10649	4884	6274	4412	4841	5264	4889	6013	3591	3719	5672	9555
	FDA007N	2820	1979	5676	2802	3199	2811	1776	2789	2503	3205	2060	1897	3911	3864
	FDA007O	5663	5198	7950	6510	10223	6400	1874	5731	7220	4489	4786	6090	8802	4313
	FDA007P	3573	2879	6983	4723	4428	3775	1312	3656	3998	3458	4330	2638	5288	2963
	S17	146	86	253	150	203	IR	99	137	108	125	138	126	IR	200
	S18	152	93	322	149	160	151	114	147	115	160	136	99	186	230
	S19	210	146	443	190	274	135	190	218	183	219	175	169	213	419
	S20	140	96	337	147	155	124	82	133	103	167	135	91	144	187
Pn 18C	FDA007A	2358	2612	8153	1687	1940	603	4085	2218	2052	2877	2573	1138	3051	2293
	FDA007B	186	552	479	192	155	18	292	168	475	169	292	91	69	153
	FDA007C	3185	2453	11861	2338	3178	1140	4234	2994	2319	4186	3259	1864	5072	2449
	FDA007D	14936	14729	25621	10048	8787	10255	32492	14662	13925	9042	14004	5895	43668	22066
	FDA007E	1775	2439	5040	1865	2932	308	1509	1646	2306	1779	2599	1703	1296	839
	FDA007F	454	730	1370	435	688	65	449	451	570	668	607	399	304	292
	FDA007G	1786	2713	4077	1628	1992	356	2540	1828	2118	1988	2269	1280	1660	1821
	FDA007H	688	955	2340	706	1263	96	555	692	746	1141	1061	812	399	374
	FDA007I	132	245	713	185	194	4	198	110	280	347	278	124	4	124
	FDA007J	6771	4507	16313	4711	8680	2167	14796	7161	5149	7954	7078	5373	9280	9455
	FDA007K	496	620	1942	488	364	65	1079	552	709	1059	733	226	297	753
	FDA007L	321	397	807	464	462	38	414	325	309	440	697	286	149	289
	FDA007M	647	1491	3390	1022	1061	4	3204	506	1159	1848	1399	657	4	2072
	FDA007N	6995	8027	19997	5796	9663	1538	8471	6889	6238	10903	7931	6235	6647	4770
	FDA007O	1954	2125	4584	1967	2723	439	2426	2022	1983	2499	2691	1577	2063	1577
	FDA007P	670	771	1561	699	1162	110	841	732	720	1090	957	673	485	610
	S21	7	4	119	4	4	4	4	7	4	83	4	4	4	4
	S22	16	38	53	21	14	4	6	13	36	37	26	8	4	5
	S23	10	4	52	18	11	4	7	9	4	36	22	6	4	6
	S24	29	40	43	45	42	4	46	24	31	30	55	25	4	31

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			Lab A	Lab B	Lab C	Lab D	Lab E	Lab F		Lab A	Lab B	Lab C	Lab D	Lab E	Lab F
Pn 19A	FDA007A	1318	655	4805	2090	1570	NT	385	1252	848	2205	1367	883	NT	1363
	FDA007B	726	467	2859	940	858	NT	188	693	605	1312	615	483	NT	678
	FDA007C	841	664	1973	1171	1024	NT	267	771	769	906	766	576	NT	886
	FDA007D	3868	2283	12076	4156	4228	NT	1786	3519	2644	5541	2718	2379	NT	5696
	FDA007E	306	353	2527	668	674	NT	7	249	415	1160	437	430	NT	11
	FDA007F	151	129	676	277	271	NT	12	137	175	305	179	173	NT	29
	FDA007G	1271	915	2492	1886	2354	NT	328	1221	1294	1124	1220	1326	NT	1151
	FDA007H	669	595	1732	952	998	NT	137	638	841	781	616	562	NT	467
	FDA007I	110	80	497	190	221	NT	10	97	101	224	123	125	NT	25
	FDA007J	993	728	3877	1308	1304	NT	201	951	962	1749	846	713	NT	765
	FDA007K	437	297	1241	533	529	NT	153	410	393	597	315	289	NT	543
	FDA007L	561	361	1578	772	771	NT	164	546	574	759	455	421	NT	581
	FDA007M	1674	1180	3728	2016	2899	NT	511	1670	1878	1792	1190	1585	NT	2048
	FDA007N	1421	726	4872	1835	1488	NT	601	1388	1155	2342	1083	813	NT	2160
	FDA007O	2177	1277	10959	3170	2268	NT	486	2171	1885	5267	1871	1501	NT	1728
	FDA007P	1529	864	5059	2348	1722	NT	474	1525	1274	1954	1609	1140	NT	1804
	S17	57	49	241	113	110	NT	4	40	58	93	78	63	NT	4
	S18	90	64	295	134	122	NT	19	77	76	114	92	70	NT	50
	S19	88	68	227	115	124	NT	24	81	80	88	79	71	NT	87
S20	50	37	295	83	85	NT	4	36	48	114	57	48	NT	4	
Pn 19F	FDA007A	1968	3513	3993	1092	1825	682	3027	1910	2534	3969	1558	966	1583	2009
	FDA007B	681	1551	1656	400	585	127	1313	632	1119	1646	570	310	230	842
	FDA007C	583	1213	1280	618	997	61	608	613	893	1273	875	527	175	488
	FDA007D	2750	4461	3701	1685	3397	1383	3259	2671	3285	3679	2385	1797	2769	2545
	FDA007E	5245	7034	12721	3041	7460	2331	4400	5157	5180	12646	4305	3885	5192	3310
	FDA007F	132	347	250	179	216	10	155	120	252	242	254	113	13	128
	FDA007G	2292	2637	4134	1793	3438	886	2434	2260	1917	4009	2539	1901	1709	2089
	FDA007H	623	1016	1013	618	1092	112	748	616	739	982	831	604	217	681
	FDA007I	626	1124	1443	496	738	142	712	604	760	1399	667	408	279	599
	FDA007J	1008	1640	2252	699	1508	286	946	950	1109	2184	939	780	511	806
	FDA007K	234	430	386	222	363	37	327	230	291	366	298	188	79	313
	FDA007L	186	364	236	250	358	21	260	186	251	224	336	185	46	257
	FDA007M	1497	2132	1689	1173	2251	688	1723	1445	1469	1602	1669	1164	1337	1466
	FDA007N	2831	3662	4257	1725	3125	1553	3943	2669	2522	4039	2454	1615	3019	2977
	FDA007O	522	1411	745	427	508	138	622	522	1139	707	607	266	251	587
	FDA007P	894	1297	1050	722	1310	284	1399	902	1047	1144	1027	685	571	1093
	S21	7	4	4	6	18	4	18	6	4	4	6	9	4	19
	S22	6	4	34	4	4	4	4	6	4	37	4	4	4	4
	S23	26	53	29	41	42	4	31	24	41	32	58	21	4	33
S24	5	4	11	4	4	4	5	5	4	13	4	4	4	5	

Pn, pneumococcal serotype; OI, opsonic index; GMOI, geometric mean opsonic index

Supplementary Table S1 (continued). Test sera OIs. The consensus and the laboratory-specific OIs for the unadjusted and normalized results are shown for each serum tested in this study. Results identified as outliers are indicated in red. Outliers were included in the determination of the consensus values in this table, but are excluded in other indicated analyses.

Serotype	Sample	Unadjusted							Normalized						
		Consensus OI	Laboratory-Specific GMOI						Consensus OI	Laboratory-Specific GMOI					
			Lab A	Lab B	Lab C	Lab D	Lab E	Lab F		Lab A	Lab B	Lab C	Lab D	Lab E	Lab F
Pn 23F	FDA007A	94	270	307	IR	9	2375	4	69	297	137	IR	13	774	4
	FDA007B	3201	2326	9834	1768	1715	16121	967	3066	2833	4382	2808	2116	5859	1956
	FDA007C	154	257	504	213	159	788	4	129	352	225	319	197	227	4
	FDA007D	939	684	1632	701	912	2590	370	887	936	727	1047	1125	777	767
	FDA007E	3067	2754	7217	1538	1939	10307	1361	2887	3771	3216	2299	2340	2975	2990
	FDA007F	61	98	193	112	104	4	58	70	101	109	168	126	4	123
	FDA007G	6423	4362	11565	4694	7226	13443	3053	6240	4471	6560	7016	8478	4292	7562
	FDA007H	581	589	967	434	495	1856	168	559	604	549	693	580	581	380
	FDA007I	275	457	855	416	561	1192	4	232	506	485	663	658	360	4
	FDA007J	1100	930	2595	584	733	3195	532	1086	1061	1472	931	924	1029	1194
	FDA007K	989	672	2169	689	1184	1435	549	1051	880	1239	1100	1493	498	1502
	FDA007L	70	116	213	131	111	4	77	90	179	122	209	139	4	210
	FDA007M	878	493	1699	604	768	3000	394	957	846	971	974	968	970	1024
	FDA007N	840	447	1170	506	678	4263	458	910	687	669	815	854	1492	1205
	FDA007O	206	416	765	272	96	2263	4	179	463	437	439	133	695	4
	FDA007P	1529	1425	2313	1120	1373	4920	513	1627	1562	1835	1804	1833	1496	1293
	S21	4	4	4	4	4	4	4	4	4	4	4	4	4	4
S22	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
S23	108	90	128	120	119	213	47	109	106	102	187	124	46	130	
S24	4	4	4	4	4	4	4	4	4	4	4	4	4	4	

Pn, pneumococcal serotype; OI, opsonic index; GMOI, geometric mean opsonic index; IR, irregular (Lab C reported IR for each of the five runs for FDA007A, serotype 23F)

Supplementary Table S2. The effect of normalization on individual samples. The overall reduction in variability due to normalization is shown for each test sample. Estimates of various variance components and CVs from the ANOVA are also shown. Outliers are included in these analyses.

Serotype	Sample	Unadjusted					Normalized					Reduction In Variation (%)
		Estimate	95% CI	Variance Estimates		%CV	Estimate	95% CI	Variance Estimates		%CV	
				Lab	Run(Lab)				Lab	Run(Lab)		
Pn1	FDA007A	311	(174, 555)	0.2971	0.0339	78%	309	(262, 365)	0.0129	0.0607	31%	77.8%
	FDA007B	272	(145, 512)	0.3451	0.0815	92%	264	(210, 332)	0.0246	0.1125	45%	67.9%
	FDA007C	266	(137, 515)	0.3845	0.0637	95%	251	(216, 292)	0.0051	0.0797	34%	81.1%
	FDA007D	434	(219, 859)	0.4073	0.0762	100%	412	(248, 684)	0.2109	0.1102	76%	33.6%
	FDA007E	72	(10, 496)	3.3602	0.1736	555%	65	(11, 374)	2.7294	0.1918	452%	17.3%
	FDA007F	77	(36, 161)	0.4976	0.0427	109%	79	(54, 114)	0.1197	0.0464	50%	69.3%
	FDA007G	1709	(1071, 2726)	0.1855	0.0623	65%	1777	(1304, 2420)	0.0751	0.0587	44%	46.0%
	FDA007H	123	(58, 259)	0.4955	0.0603	111%	128	(86, 190)	0.1347	0.0414	52%	68.3%
	FDA007I	117	(55, 248)	0.4976	0.0815	114%	118	(90, 157)	0.0536	0.0864	45%	75.8%
	FDA007J	110	(63, 192)	0.2697	0.0771	80%	111	(95, 130)	0.0053	0.0828	35%	74.6%
	FDA007K	234	(131, 419)	0.2930	0.0665	82%	247	(194, 315)	0.0427	0.0524	36%	73.5%
	FDA007L	141	(65, 306)	0.5335	0.0721	118%	146	(102, 211)	0.1107	0.0573	51%	72.3%
	FDA007M	10	(2, 41)	1.8149	0.0776	296%	8	(3, 24)	1.0336	0.1267	194%	38.7%
	FDA007N	148	(66, 331)	0.5602	0.1305	130%	157	(111, 220)	0.0748	0.1468	60%	67.9%
	FDA007O	592	(347, 1009)	0.2457	0.0666	75%	618	(471, 810)	0.0514	0.0766	43%	59.0%
	FDA007P	270	(169, 429)	0.1926	0.0197	59%	275	(216, 352)	0.0393	0.0738	40%	46.7%
	S21	4	(4, 5)	0.0000	0.0507	25%	4	(4, 4)	0.0000	0.0191	15%	62.4%
S22	5	(3, 6)	0.0578	0.0733	44%	5	(3, 6)	0.0672	0.0911	49%	-17.2%	
S23	14	(5, 36)	0.7861	0.1326	161%	15	(5, 45)	1.0160	0.1205	190%	-19.2%	
S24	6	(3, 14)	0.5753	0.0355	118%	6	(3, 12)	0.4089	0.0668	99%	22.1%	
Pn3	FDA007A	69	(28, 173)	0.7311	0.1122	151%	64	(30, 140)	0.5281	0.0969	120%	25.9%
	FDA007B	66	(21, 208)	1.1603	0.1545	215%	61	(24, 155)	0.7552	0.1316	156%	32.6%
	FDA007C	30	(18, 49)	0.2178	0.0583	69%	29	(25, 33)	0.0000	0.0970	37%	64.9%
	FDA007D	190	(79, 457)	0.6769	0.0911	140%	184	(104, 326)	0.2779	0.0979	85%	51.1%
	FDA007E	94	(24, 366)	1.6153	0.2194	287%	86	(33, 224)	0.7811	0.2567	177%	43.4%
	FDA007F	38	(23, 61)	0.1965	0.0819	69%	35	(30, 41)	0.0000	0.1009	37%	63.8%
	FDA007G	313	(203, 483)	0.1587	0.0630	60%	309	(255, 375)	0.0172	0.0839	37%	54.4%
	FDA007H	48	(23, 101)	0.4820	0.1015	115%	47	(31, 71)	0.1476	0.0649	59%	63.6%
	FDA007I	32	(12, 80)	0.7706	0.0879	153%	29	(15, 55)	0.3380	0.1440	100%	43.9%
	FDA007J	12	(4, 38)	1.2161	0.1563	223%	10	(3, 31)	1.1559	0.0912	205%	9.1%
	FDA007K	44	(22, 87)	0.3903	0.1429	108%	39	(27, 57)	0.0862	0.1686	66%	52.2%
	FDA007L	58	(32, 107)	0.3187	0.0891	89%	54	(46, 64)	0.0100	0.0693	33%	80.6%
	FDA007M	96	(42, 220)	0.6085	0.0688	128%	87	(60, 127)	0.1066	0.1154	60%	67.2%
	FDA007N	305	(171, 545)	0.2879	0.0846	84%	281	(239, 331)	0.0029	0.1073	39%	70.4%
	FDA007O	134	(73, 248)	0.3230	0.0979	91%	123	(84, 180)	0.1150	0.0784	55%	54.0%
	FDA007P	196	(89, 433)	0.5580	0.0530	119%	189	(132, 270)	0.0944	0.1061	56%	67.2%
	S21	4	(4, 4)	0.0000	0.0000	0%	4	(4, 4)	0.0000	0.0000	0%	NA
S22	4	(4, 5)	0.0127	0.0436	27%	4	(4, 4)	0.0019	0.0073	10%	83.6%	
S23	5	(3, 6)	0.0756	0.0667	46%	4	(4, 5)	0.0027	0.0117	13%	89.9%	
S24	4	(4, 4)	0.0000	0.0000	0%	4	(4, 4)	0.0000	0.0000	0%	NA	

Pn, pneumococcal serotype; **CI**, confidence interval; **CV**, coefficient of variation (expressed as a percent); **Lab**, variability among the laboratories; **Run(Lab)**, variability among the runs within each laboratory.

Supplementary Table S2 (continued). The effect of normalization on individual samples. The overall reduction in variability due to normalization is shown for each test sample. Estimates of various variance components and CVs from the ANOVA are also shown. Outliers are included in these analyses.

Serotype	Sample	Unadjusted					Normalized					Reduction In Variation (%)
		Estimate	95% CI	Variance Estimates		%CV	Estimate	95% CI	Variance Estimates		%CV	
				Lab	Run(Lab)				Lab	Run(Lab)		
Pn4	FDA007A	5157	(2634, 10099)	0.3920	0.0902	100%	5371	(4262, 6768)	0.0318	0.0809	40%	76.6%
	FDA007B	1542	(874, 2721)	0.2769	0.0777	81%	1617	(1437, 1821)	0.0004	0.0598	28%	83.0%
	FDA007C	3848	(1963, 7544)	0.3997	0.0580	97%	3890	(2582, 5862)	0.1343	0.0881	60%	51.4%
	FDA007D	10515	(5658, 19545)	0.3305	0.0917	92%	10815	(8833, 13240)	0.0176	0.0944	40%	73.5%
	FDA007E	3955	(1725, 9070)	0.6025	0.1093	132%	4118	(2755, 6155)	0.1234	0.1072	62%	67.6%
	FDA007F	396	(207, 760)	0.3647	0.0978	97%	430	(312, 593)	0.0651	0.1320	56%	57.4%
	FDA007G	1822	(1152, 2881)	0.1761	0.0730	65%	1949	(1441, 2637)	0.0745	0.0409	40%	53.7%
	FDA007H	1350	(843, 2162)	0.1814	0.0988	70%	1462	(1272, 1680)	0.0000	0.0853	34%	69.6%
	FDA007I	610	(389, 957)	0.1607	0.1172	69%	653	(566, 752)	0.0075	0.0523	28%	78.5%
	FDA007J	1189	(649, 2178)	0.3013	0.1408	94%	1205	(995, 1458)	0.0196	0.0601	33%	82.0%
	FDA007K	820	(516, 1302)	0.1878	0.0318	60%	799	(584, 1094)	0.0721	0.0845	49%	28.7%
	FDA007L	1440	(773, 2681)	0.3239	0.1347	97%	1459	(1109, 1921)	0.0438	0.1194	50%	64.4%
	FDA007M	2873	(1696, 4866)	0.2320	0.0906	76%	2760	(1877, 4059)	0.1028	0.1414	64%	24.3%
	FDA007N	4163	(2368, 7321)	0.2725	0.0836	82%	4257	(2990, 6062)	0.0958	0.0850	53%	49.3%
	FDA007O	7735	(4559, 13123)	0.2389	0.0744	75%	8089	(7258, 9015)	0.0005	0.0492	25%	84.1%
	FDA007P	2970	(1639, 5381)	0.3033	0.0839	86%	3023	(2632, 3471)	0.0000	0.0810	33%	79.1%
	S17	84	(16, 432)	2.4342	0.0226	379%	77	(17, 361)	2.1349	0.0519	339%	11.0%
	S18	96	(17, 529)	2.6355	0.0855	420%	89	(17, 461)	2.4432	0.0952	392%	6.7%
	S19	60	(13, 276)	2.1073	0.0662	337%	58	(14, 230)	1.7179	0.0869	283%	17.0%
	S20	85	(16, 449)	2.4824	0.0652	393%	86	(17, 423)	2.3041	0.0453	363%	7.8%
Pn5	FDA007A	489	(262, 915)	0.3367	0.0911	92%	475	(338, 670)	0.0810	0.1228	57%	52.4%
	FDA007B	329	(204, 530)	0.1851	0.1117	72%	324	(218, 482)	0.1176	0.1185	63%	20.5%
	FDA007C	364	(209, 634)	0.2681	0.0605	77%	345	(281, 423)	0.0218	0.0783	37%	69.5%
	FDA007D	1513	(921, 2487)	0.2172	0.0337	65%	1458	(1085, 1959)	0.0688	0.0499	41%	52.7%
	FDA007E	153	(74, 313)	0.4548	0.0647	106%	145	(95, 222)	0.1505	0.0577	58%	59.9%
	FDA007F	223	(159, 313)	0.0960	0.0386	44%	221	(165, 296)	0.0564	0.0988	48%	-13.3%
	FDA007G	298	(253, 351)	0.0162	0.0391	27%	305	(218, 427)	0.0869	0.0760	50%	-66.0%
	FDA007H	337	(141, 807)	0.6766	0.0848	139%	351	(192, 645)	0.3162	0.0884	89%	46.9%
	FDA007I	95	(55, 163)	0.2489	0.0827	78%	93	(63, 136)	0.1117	0.1003	58%	36.0%
	FDA007J	10	(5, 20)	0.4672	0.0512	105%	10	(5, 21)	0.5104	0.0933	117%	-14.1%
	FDA007K	2265	(954, 5376)	0.6746	0.0191	130%	2343	(1320, 4157)	0.2894	0.0441	78%	51.9%
	FDA007L	72	(35, 147)	0.4477	0.0849	107%	74	(45, 121)	0.2086	0.0807	71%	45.7%
	FDA007M	144	(70, 297)	0.4601	0.0891	110%	155	(96, 253)	0.1918	0.1081	73%	45.4%
	FDA007N	2279	(1244, 4173)	0.3238	0.0436	83%	2447	(1801, 3324)	0.0767	0.0412	41%	67.9%
	FDA007O	118	(52, 268)	0.5751	0.1654	136%	123	(60, 252)	0.4339	0.1831	119%	16.7%
	FDA007P	498	(268, 925)	0.3342	0.0663	88%	525	(365, 755)	0.1091	0.0524	49%	59.7%
	S21	5	(3, 6)	0.1024	0.0016	38%	4	(4, 5)	0.0304	0.0053	21%	65.6%
	S22	4	(4, 4)	0.0000	0.0000	0%	4	(4, 4)	0.0000	0.0000	0%	NA
	S23	4	(4, 4)	0.0000	0.0000	0%	4	(4, 4)	0.0000	0.0000	0%	NA
	S24	4	(4, 4)	0.0000	0.0000	0%	4	(4, 4)	0.0000	0.0000	0%	NA

Pn, pneumococcal serotype; CI, confidence interval; CV, coefficient of variation (expressed as a percent); Lab, variability among the laboratories; Run(Lab), variability among the runs within each laboratory.

Supplementary Table S2 (continued). The effect of normalization on individual samples. The overall reduction in variability due to normalization is shown for each test sample. Estimates of various variance components and CVs from the ANOVA are also shown. Outliers are included in these analyses.

Serotype	Sample	Unadjusted					Normalized					Reduction In Variation (%)
		Estimate	95% CI	Variance Estimates		%CV	Estimate	95% CI	Variance Estimates		%CV	
				Lab	Run(Lab)				Lab	Run(Lab)		
Pn6A	FDA007A	1316	(803, 2156)	0.1982	0.1160	75%	1270	(884, 1825)	0.1057	0.0655	51%	45.5%
	FDA007B	1104	(660, 1848)	0.2028	0.1908	87%	1069	(798, 1433)	0.0594	0.0887	47%	62.4%
	FDA007C	795	(515, 1228)	0.1485	0.1115	67%	717	(519, 991)	0.0811	0.0650	47%	43.8%
	FDA007D	19974	(12900, 30927)	0.1477	0.1293	69%	20197	(13192, 30924)	0.1401	0.1182	66%	6.7%
	FDA007E	2906	(1721, 4905)	0.2314	0.0878	76%	2803	(2192, 3585)	0.0386	0.0787	41%	63.3%
	FDA007F	1534	(949, 2480)	0.1959	0.0675	67%	1460	(1107, 1924)	0.0508	0.0891	45%	46.9%
	FDA007G	2930	(1670, 5142)	0.2715	0.0784	81%	2895	(1793, 4674)	0.1862	0.1063	72%	16.4%
	FDA007H	1576	(980, 2536)	0.1928	0.0634	66%	1510	(1051, 2170)	0.1051	0.0682	52%	32.3%
	FDA007I	375	(272, 518)	0.0590	0.1769	63%	390	(302, 502)	0.0419	0.0787	42%	48.9%
	FDA007J	1400	(979, 2001)	0.0944	0.1075	57%	1397	(1102, 1772)	0.0291	0.1066	45%	32.8%
	FDA007K	362	(254, 517)	0.0632	0.2601	77%	362	(290, 452)	0.0176	0.1299	47%	54.4%
	FDA007L	126	(24, 650)	2.4236	0.1268	394%	116	(22, 613)	2.4880	0.1730	411%	-4.2%
	FDA007M	2444	(1878, 3180)	0.0423	0.1034	46%	2413	(2128, 2737)	0.0000	0.0696	30%	52.2%
	FDA007N	619	(346, 1108)	0.2895	0.0873	85%	623	(373, 1041)	0.2260	0.0636	71%	23.1%
	FDA007O	1105	(772, 1583)	0.0987	0.0923	55%	1172	(919, 1495)	0.0436	0.0479	35%	52.1%
	FDA007P	5047	(3191, 7984)	0.1749	0.0801	66%	5171	(3888, 6879)	0.0605	0.0648	42%	50.9%
	S21	6	(2, 15)	0.7840	0.1037	157%	6	(2, 13)	0.5759	0.1520	135%	18.0%
S22	46	(28, 77)	0.2197	0.0782	73%	48	(27, 86)	0.2933	0.0721	83%	-18.5%	
S23	25	(11, 57)	0.6036	0.1719	141%	26	(10, 65)	0.7018	0.3222	175%	-24.3%	
S24	9	(3, 26)	0.9660	0.1747	191%	8	(3, 21)	0.7724	0.0972	154%	23.8%	
Pn6B	FDA007A	789	(468, 1330)	0.2222	0.1281	81%	763	(500, 1165)	0.1212	0.1977	76%	9.0%
	FDA007B	861	(528, 1404)	0.2005	0.0811	70%	831	(681, 1014)	0.0100	0.1253	44%	51.9%
	FDA007C	2225	(1393, 3555)	0.1826	0.0842	68%	2264	(1598, 3207)	0.0915	0.0894	53%	32.2%
	FDA007D	13018	(7521, 22534)	0.2533	0.1003	81%	13346	(10226, 17420)	0.0451	0.0931	45%	60.9%
	FDA007E	3400	(2194, 5268)	0.1613	0.0646	61%	3561	(2906, 4362)	0.0218	0.0754	37%	57.0%
	FDA007F	1479	(850, 2575)	0.2627	0.0806	80%	1646	(1151, 2354)	0.1019	0.0684	51%	50.4%
	FDA007G	5930	(2823, 12454)	0.4905	0.0481	108%	6066	(3204, 11483)	0.3603	0.0456	89%	24.6%
	FDA007H	1313	(858, 2009)	0.1510	0.0666	59%	1368	(961, 1947)	0.0989	0.0686	51%	23.0%
	FDA007I	423	(272, 658)	0.1590	0.0729	62%	397	(340, 464)	0.0000	0.0983	37%	57.6%
	FDA007J	2068	(1366, 3131)	0.1362	0.1000	63%	1906	(1601, 2269)	0.0081	0.0940	38%	56.8%
	FDA007K	651	(407, 1040)	0.1907	0.0436	62%	610	(490, 761)	0.0268	0.0835	39%	52.9%
	FDA007L	182	(35, 941)	2.4239	0.0815	387%	155	(24, 989)	3.0703	0.1129	495%	-21.3%
	FDA007M	4247	(2376, 7591)	0.2781	0.1413	91%	4186	(2921, 5998)	0.1010	0.0793	53%	57.0%
	FDA007N	1975	(1106, 3524)	0.2820	0.1142	88%	1923	(1260, 2937)	0.1389	0.1147	65%	36.0%
	FDA007O	7497	(4369, 12863)	0.2536	0.0550	74%	7438	(4562, 12127)	0.2078	0.0442	65%	18.4%
	FDA007P	6680	(4167, 10709)	0.1909	0.0563	64%	6526	(4930, 8641)	0.0580	0.0650	42%	50.2%
	S17	85	(17, 433)	2.3958	0.0618	380%	77	(17, 354)	2.0825	0.1192	341%	10.4%
S18	151	(79, 285)	0.2419	0.1166	82%	141	(110, 179)	0.0124	0.1278	45%	60.9%	
S19	174	(99, 306)	0.2630	0.0930	82%	183	(161, 208)	0.0000	0.0642	29%	82.0%	
S20	79	(17, 375)	2.2032	0.0569	350%	74	(17, 333)	2.0173	0.0905	327%	6.7%	

Pn, pneumococcal serotype; CI, confidence interval; CV, coefficient of variation (expressed as a percent); Lab, variability among the laboratories; Run(Lab), variability among the runs within each laboratory.

Supplementary Table S2 (continued). The effect of normalization on individual samples. The overall reduction in variability due to normalization is shown for each test sample. Estimates of various variance components and CVs from the ANOVA are also shown. Outliers are included in these analyses.

Serotype	Sample	Unadjusted					Normalized					Reduction In Variation (%)
		Estimate	95% CI	Variance Estimates		%CV	Estimate	95% CI	Variance Estimates		%CV	
				Lab	Run(Lab)				Lab	Run(Lab)		
Pn7F	FDA007A	7211	(2851, 18233)	0.7729	0.0432	147%	7066	(4403, 11341)	0.1940	0.0444	63%	70.8%
	FDA007B	7978	(3104, 20498)	0.7942	0.0727	154%	8157	(5438, 12236)	0.1359	0.0641	56%	76.9%
	FDA007C	8464	(4475, 16012)	0.3553	0.0684	92%	8422	(7384, 9606)	0.0000	0.0759	32%	82.1%
	FDA007D	21335	(12351, 36853)	0.2365	0.1738	90%	22290	(17233, 28834)	0.0217	0.1856	58%	49.5%
	FDA007E	8781	(3612, 21348)	0.7053	0.0561	139%	9105	(6890, 12033)	0.0557	0.0716	43%	83.3%
	FDA007F	2698	(1148, 6340)	0.6501	0.0628	133%	2555	(1877, 3478)	0.0623	0.1160	53%	75.0%
	FDA007G	10318	(3936, 27043)	0.8363	0.0345	154%	10171	(6628, 15609)	0.1500	0.0792	61%	73.7%
	FDA007H	2634	(1289, 5384)	0.4545	0.0473	103%	2550	(2123, 3062)	0.0122	0.0878	37%	80.1%
	FDA007I	3877	(1553, 9676)	0.7429	0.0841	148%	3757	(2758, 5117)	0.0602	0.1277	54%	77.3%
	FDA007J	2056	(823, 5137)	0.7410	0.1040	151%	1942	(1347, 2800)	0.0988	0.1096	58%	75.3%
	FDA007K	904	(422, 1939)	0.5149	0.0655	114%	917	(699, 1204)	0.0515	0.0744	43%	78.3%
	FDA007L	2619	(1276, 5374)	0.4459	0.1173	112%	2570	(2088, 3163)	0.0189	0.0976	41%	79.3%
	FDA007M	5594	(2429, 12883)	0.6162	0.0713	129%	5450	(4137, 7179)	0.0498	0.0842	44%	80.5%
	FDA007N	6884	(3164, 14980)	0.5274	0.1077	122%	6947	(4811, 10030)	0.0960	0.1277	60%	64.8%
	FDA007O	3952	(1747, 8937)	0.5761	0.1430	133%	3947	(2798, 5567)	0.0934	0.0675	49%	77.6%
	FDA007P	2418	(1104, 5295)	0.5324	0.1267	125%	2384	(1862, 3053)	0.0317	0.1143	47%	77.8%
	S21	72	(23, 222)	1.1310	0.1384	209%	63	(27, 148)	0.5805	0.3474	162%	26.9%
S22	68	(18, 259)	1.5883	0.1496	274%	60	(23, 155)	0.7544	0.2911	178%	39.8%	
S23	130	(67, 255)	0.3892	0.0911	100%	126	(86, 184)	0.1066	0.1270	62%	51.4%	
S24	75	(25, 221)	1.0220	0.2172	204%	72	(35, 145)	0.3858	0.3129	131%	43.6%	
Pn9V	FDA007A	8502	(5465, 13227)	0.1508	0.1327	70%	7771	(5943, 10163)	0.0534	0.0576	40%	60.9%
	FDA007B	2753	(1382, 5486)	0.4030	0.1420	109%	2495	(1653, 3765)	0.1429	0.0525	56%	64.1%
	FDA007C	1161	(658, 2048)	0.2718	0.1039	85%	1010	(639, 1595)	0.1728	0.0817	66%	32.2%
	FDA007D	9509	(5713, 15826)	0.2178	0.0893	74%	8591	(4927, 14980)	0.2620	0.0899	81%	-12.7%
	FDA007E	5874	(3593, 9603)	0.1829	0.1822	83%	5067	(3835, 6697)	0.0582	0.0594	41%	67.8%
	FDA007F	966	(687, 1358)	0.0802	0.1258	57%	836	(639, 1094)	0.0501	0.0749	42%	39.3%
	FDA007G	2825	(1819, 4388)	0.1606	0.0777	63%	2477	(2169, 2830)	0.0000	0.0776	32%	67.4%
	FDA007H	882	(473, 1642)	0.3217	0.1470	98%	777	(528, 1142)	0.1048	0.1457	65%	46.6%
	FDA007I	896	(652, 1230)	0.0729	0.0923	50%	829	(502, 1371)	0.1971	0.1567	81%	-53.3%
	FDA007J	2676	(1602, 4471)	0.2122	0.1340	80%	2397	(1829, 3141)	0.0398	0.1280	51%	51.5%
	FDA007K	1533	(905, 2597)	0.2353	0.0863	76%	1477	(947, 2302)	0.1606	0.0883	65%	22.6%
	FDA007L	2246	(957, 5268)	0.6406	0.0976	136%	2119	(1115, 4024)	0.3571	0.0795	94%	40.9%
	FDA007M	1268	(841, 1913)	0.1387	0.0727	58%	1195	(958, 1492)	0.0336	0.0523	34%	59.4%
	FDA007N	18983	(10684, 33729)	0.2824	0.0878	84%	16503	(11321, 24055)	0.1041	0.1193	60%	39.6%
	FDA007O	1621	(1145, 2295)	0.0909	0.0945	54%	1507	(744, 3056)	0.4381	0.0733	104%	-63.7%
	FDA007P	2310	(1548, 3446)	0.1265	0.0947	60%	2154	(1483, 3128)	0.1032	0.1111	59%	3.1%
	S21	72	(21, 243)	1.3008	0.2126	242%	69	(25, 187)	0.8383	0.3622	199%	20.7%
S22	7	(3, 17)	0.7216	0.0643	143%	7	(3, 19)	0.7784	0.1669	164%	-16.9%	
S23	23	(8, 69)	1.0325	0.1485	196%	22	(8, 65)	0.9701	0.2986	208%	-6.9%	
S24	14	(5, 38)	0.8460	0.3989	205%	15	(5, 44)	0.9691	0.4252	226%	-10.7%	

Pn, pneumococcal serotype; **CI**, confidence interval; **CV**, coefficient of variation (expressed as a percent); **Lab**, variability among the laboratories; **Run(Lab)**, variability among the runs within each laboratory.

Supplementary Table S2 (continued). The effect of normalization on individual samples. The overall reduction in variability due to normalization is shown for each test sample. Estimates of various variance components and CVs from the ANOVA are also shown. Outliers are included in these analyses.

Serotype	Sample	Unadjusted					Normalized					Reduction In Variation (%)
		Estimate	95% CI	Variance Estimates		%CV	Estimate	95% CI	Variance Estimates		%CV	
				Lab	Run(Lab)				Lab	Run(Lab)		
Pn14	FDA007A	2326	(1726, 3135)	0.0720	0.0443	41%	2289	(1680, 3120)	0.0783	0.0418	41%	-3.2%
	FDA007B	9299	(5091, 16985)	0.3189	0.0531	84%	9290	(6915, 12480)	0.0726	0.0315	38%	72.0%
	FDA007C	780	(580, 1048)	0.0675	0.0595	43%	766	(545, 1075)	0.0876	0.0784	50%	-23.5%
	FDA007D	4369	(2886, 6614)	0.1396	0.0829	60%	4339	(3590, 5243)	0.0215	0.0513	31%	67.3%
	FDA007E	9072	(5004, 16449)	0.3089	0.0628	84%	9051	(6450, 12699)	0.0899	0.0658	48%	58.1%
	FDA007F	888	(571, 1380)	0.1674	0.0459	59%	953	(748, 1214)	0.0433	0.0472	35%	57.6%
	FDA007G	7439	(3839, 14416)	0.3815	0.0801	97%	7713	(5942, 10014)	0.0515	0.0498	37%	78.1%
	FDA007H	998	(643, 1550)	0.1611	0.0742	62%	1041	(737, 1472)	0.0966	0.0571	48%	34.7%
	FDA007I	1579	(909, 2743)	0.2512	0.1291	85%	1567	(1215, 2022)	0.0450	0.0667	40%	70.6%
	FDA007J	1276	(838, 1944)	0.1434	0.0872	62%	1295	(960, 1747)	0.0704	0.0528	42%	46.6%
	FDA007K	549	(379, 795)	0.1071	0.0872	55%	530	(416, 676)	0.0442	0.0435	34%	54.9%
	FDA007L	516	(338, 788)	0.1464	0.0839	62%	515	(375, 708)	0.0758	0.0746	47%	34.7%
	FDA007M	5489	(3763, 8006)	0.1056	0.1187	61%	5264	(3594, 7710)	0.1180	0.0662	54%	17.9%
	FDA007N	2820	(1832, 4340)	0.1493	0.0979	64%	2789	(2005, 3879)	0.0862	0.0588	46%	41.3%
	FDA007O	5663	(3056, 10497)	0.3374	0.0419	85%	5731	(4247, 7732)	0.0712	0.0496	42%	68.2%
	FDA007P	3573	(1962, 6506)	0.3169	0.0441	82%	3656	(2814, 4751)	0.0514	0.0493	37%	72.1%
	S17	146	(82, 258)	0.2005	0.0596	67%	137	(103, 182)	0.0423	0.0521	36%	63.7%
	S18	152	(97, 238)	0.1628	0.0941	66%	147	(106, 205)	0.0789	0.0838	50%	36.7%
	S19	210	(132, 334)	0.1806	0.0689	65%	218	(152, 311)	0.1014	0.0680	51%	32.1%
	S20	140	(83, 236)	0.2307	0.0833	75%	133	(99, 179)	0.0689	0.0457	40%	63.5%
Pn18C	FDA007A	2358	(938, 5929)	0.7302	0.2098	164%	2218	(1522, 3233)	0.1007	0.1354	63%	74.9%
	FDA007B	186	(50, 688)	1.5078	0.2349	274%	168	(79, 357)	0.4727	0.1928	126%	61.8%
	FDA007C	3185	(1407, 7208)	0.5652	0.2035	140%	2994	(2012, 4454)	0.1228	0.0990	60%	71.1%
	FDA007D	14936	(8446, 26413)	0.2665	0.1428	90%	14662	(7071, 30403)	0.4677	0.0732	109%	-24.3%
	FDA007E	1775	(653, 4823)	0.8893	0.0917	169%	1646	(1069, 2533)	0.1513	0.0844	62%	76.0%
	FDA007F	454	(153, 1348)	1.0480	0.1288	196%	451	(308, 658)	0.0982	0.1549	65%	78.5%
	FDA007G	1786	(733, 4352)	0.6918	0.1450	150%	1828	(1469, 2276)	0.0214	0.1067	43%	84.7%
	FDA007H	688	(220, 2152)	1.1653	0.0725	204%	692	(419, 1143)	0.2171	0.0554	69%	78.0%
	FDA007I	132	(21, 837)	3.0494	0.2044	507%	110	(19, 627)	2.7094	0.1675	445%	11.6%
	FDA007J	6772	(2987, 15350)	0.5900	0.0914	128%	7161	(5433, 9438)	0.0537	0.0746	43%	81.2%
	FDA007K	496	(146, 1683)	1.3122	0.2111	244%	552	(292, 1044)	0.3301	0.1877	105%	66.0%
	FDA007L	321	(104, 987)	1.1264	0.0993	203%	325	(191, 555)	0.2349	0.1184	81%	71.2%
	FDA007M	647	(45, 9196)	6.3558	0.1716	1187%	506	(41, 6244)	5.7087	0.1062	1015%	10.9%
	FDA007N	6995	(2874, 17026)	0.7088	0.0488	139%	6889	(5130, 9251)	0.0575	0.1032	49%	78.8%
	FDA007O	1954	(853, 4474)	0.6041	0.0968	131%	2022	(1596, 2562)	0.0428	0.0385	33%	88.4%
	FDA007P	670	(251, 1786)	0.8624	0.0568	161%	732	(538, 995)	0.0623	0.1126	52%	81.0%
	S21	7	(2, 30)	1.9068	0.0648	307%	7	(2, 24)	1.5225	0.0631	252%	19.6%
	S22	16	(5, 46)	0.9886	0.2185	200%	13	(5, 39)	0.9492	0.2541	200%	0.3%
	S23	10	(4, 29)	0.9478	0.1336	183%	9	(3, 24)	0.8587	0.0556	160%	15.4%
	S24	29	(11, 80)	0.8678	0.1963	181%	23	(9, 59)	0.7186	0.2485	167%	9.1%

Pn, pneumococcal serotype; CI, confidence interval; CV, coefficient of variation (expressed as a percent); Lab, variability among the laboratories; Run(Lab), variability among the runs within each laboratory.

Supplementary Table S2 (continued). The effect of normalization on individual samples. The overall reduction in variability due to normalization is shown for each test sample. Estimates of various variance components and CVs from the ANOVA are also shown. Outliers are included in these analyses.

Serotype	Sample	Unadjusted					Normalized					Reduction In Variation (%)
		Estimate	95% CI	Variance Estimates		%CV	Estimate	95% CI	Variance Estimates		%CV	
				Lab	Run(Lab)				Lab	Run(Lab)		
Pn19A	FDA007A	1318	(385, 4505)	0.9595	0.1033	180%	1252	(771, 2032)	0.1416	0.0527	55%	81.7%
	FDA007B	726	(210, 2513)	0.9761	0.1155	184%	693	(433, 1108)	0.1327	0.0508	53%	83.2%
	FDA007C	841	(331, 2132)	0.5540	0.0388	116%	771	(616, 964)	0.0242	0.0417	29%	88.9%
	FDA007D	3868	(1545, 9681)	0.5305	0.0773	118%	3519	(2063, 6002)	0.1782	0.0340	59%	65.1%
	FDA007E	306	(19, 5027)	5.0493	0.1563	879%	249	(26, 2374)	3.2156	0.3902	568%	30.7%
	FDA007F	151	(23, 1011)	2.3219	0.0918	373%	137	(45, 418)	0.7679	0.2158	170%	59.2%
	FDA007G	1271	(439, 3680)	0.7211	0.0579	142%	1221	(1115, 1336)	0.0000	0.0263	18%	96.6%
	FDA007H	669	(202, 2214)	0.9186	0.0512	168%	638	(473, 861)	0.0480	0.0506	37%	89.8%
	FDA007I	110	(17, 713)	2.2314	0.1410	367%	97	(35, 268)	0.6017	0.3056	159%	61.8%
	FDA007J	993	(260, 3790)	1.1483	0.0733	202%	951	(609, 1485)	0.1178	0.0553	52%	85.8%
	FDA007K	437	(167, 1146)	0.5889	0.0670	125%	410	(275, 611)	0.0904	0.0655	48%	76.2%
	FDA007L	561	(192, 1638)	0.7297	0.0770	146%	546	(410, 728)	0.0400	0.0679	39%	86.6%
	FDA007M	1674	(627, 4472)	0.6106	0.0794	129%	1670	(1285, 2170)	0.0323	0.0610	36%	86.5%
	FDA007N	1421	(505, 4001)	0.6793	0.0776	139%	1388	(783, 2460)	0.1995	0.0651	67%	65.0%
	FDA007O	2177	(522, 9076)	1.3045	0.0883	225%	2171	(1161, 4058)	0.2368	0.0857	76%	76.8%
	FDA007P	1529	(493, 4748)	0.8229	0.0474	154%	1525	(1147, 2026)	0.0437	0.0439	34%	89.9%
	S17	57	(8, 406)	2.5112	0.0172	390%	40	(8, 203)	1.6890	0.0510	274%	31.2%
	S18	90	(25, 320)	1.0205	0.1317	193%	77	(53, 114)	0.0230	0.3636	86%	66.4%
	S19	88	(31, 250)	0.6933	0.0317	134%	81	(71, 91)	0.0000	0.0509	25%	93.0%
	S20	50	(7, 360)	2.5305	0.0449	398%	36	(7, 175)	1.6230	0.0347	262%	35.6%
Pn19F	FDA007A	1968	(939, 4126)	0.4720	0.1176	116%	1910	(1146, 3181)	0.2160	0.0893	74%	48.2%
	FDA007B	681	(238, 1953)	0.9721	0.1732	192%	632	(286, 1396)	0.5353	0.1648	131%	38.9%
	FDA007C	583	(182, 1872)	1.2098	0.0755	211%	613	(308, 1220)	0.4037	0.0823	101%	62.2%
	FDA007D	2750	(1682, 4494)	0.1994	0.0954	72%	2671	(2034, 3506)	0.0468	0.0935	45%	52.4%
	FDA007E	5245	(2709, 10154)	0.3747	0.1077	100%	5157	(3137, 8479)	0.2038	0.0990	73%	37.2%
	FDA007F	132	(34, 507)	1.5898	0.2583	289%	120	(36, 396)	1.2246	0.3367	249%	15.5%
	FDA007G	2292	(1290, 4071)	0.2906	0.0450	78%	2260	(1631, 3133)	0.0858	0.0523	45%	58.8%
	FDA007H	622	(250, 1548)	0.7376	0.0801	147%	616	(352, 1077)	0.2689	0.0717	79%	58.4%
	FDA007I	626	(266, 1474)	0.6447	0.1082	138%	604	(339, 1076)	0.2867	0.0748	82%	52.0%
	FDA007J	1008	(462, 2203)	0.5398	0.0749	119%	950	(573, 1576)	0.2190	0.0652	70%	53.8%
	FDA007K	234	(88, 618)	0.8433	0.0797	161%	230	(127, 417)	0.3086	0.0584	83%	60.2%
	FDA007L	186	(59, 584)	1.1699	0.0824	206%	186	(89, 391)	0.4746	0.1201	116%	52.5%
	FDA007M	1497	(939, 2388)	0.1805	0.0865	68%	1445	(1217, 1716)	0.0000	0.1296	43%	51.4%
	FDA007N	2830	(1788, 4481)	0.1776	0.0705	65%	2669	(1937, 3677)	0.0763	0.0818	49%	36.3%
	FDA007O	522	(234, 1161)	0.5570	0.1062	126%	522	(283, 961)	0.3190	0.0814	88%	39.6%
	FDA007P	894	(471, 1699)	0.3585	0.0791	94%	902	(670, 1215)	0.0613	0.0919	48%	65.0%
	S21	7	(3, 15)	0.5377	0.0746	119%	6	(3, 12)	0.3549	0.1188	99%	22.6%
	S22	6	(2, 14)	0.7559	0.0732	149%	6	(2, 15)	0.8105	0.1134	161%	-10.3%
	S23	26	(10, 71)	0.8922	0.0296	161%	24	(9, 66)	0.8841	0.0502	163%	-1.3%
	S24	5	(3, 8)	0.1528	0.1241	69%	5	(3, 8)	0.1825	0.1376	76%	-13.5%

Pn, pneumococcal serotype; **CI**, confidence interval; **CV**, coefficient of variation (expressed as a percent); **Lab**, variability among the laboratories; **Run(Lab)**, variability among the runs within each laboratory.

Supplementary Table S2 (continued). The effect of normalization on individual samples. The overall reduction in variability due to normalization is shown for each test sample. Estimates of various variance components and CVs from the ANOVA are also shown. Outliers are included in these analyses.

Serotype	Sample	Unadjusted					Normalized					Reduction In Variation (%)
		Estimate	95% CI	Variance Estimates		%CV	Estimate	95% CI	Variance Estimates		%CV	
				Lab	Run(Lab)				Lab	Run(Lab)		
Pn23F	FDA007A	94	(3, 2547)	7.0200	0.1165	1346%	69	(4, 1069)	4.8247	0.1512	831%	30.3%
	FDA007B	3201	(1001, 10237)	1.2083	0.0927	213%	3066	(1971, 4768)	0.1687	0.0380	58%	84.1%
	FDA007C	154	(21, 1112)	3.5017	0.1086	569%	129	(21, 778)	2.9284	0.0375	460%	17.8%
	FDA007D	939	(454, 1939)	0.4558	0.1104	112%	887	(732, 1076)	0.0091	0.1188	43%	77.4%
	FDA007E	3067	(1265, 7434)	0.6921	0.0993	143%	2887	(2361, 3531)	0.0295	0.0351	29%	91.8%
	FDA007F	61	(14, 262)	1.8947	0.1366	316%	70	(16, 307)	1.9702	0.0806	319%	-1.0%
	FDA007G	6423	(3479, 11857)	0.3064	0.1746	100%	6240	(4667, 8343)	0.0374	0.1889	61%	52.9%
	FDA007H	581	(250, 1353)	0.6237	0.1175	137%	559	(456, 685)	0.0199	0.0816	38%	86.3%
	FDA007I	275	(30, 2523)	4.4194	0.1826	754%	232	(28, 1893)	3.9824	0.1193	658%	10.9%
	FDA007J	1099	(488, 2477)	0.5773	0.1043	128%	1086	(900, 1312)	0.0207	0.0535	31%	89.1%
	FDA007K	989	(566, 1728)	0.2649	0.0898	81%	1051	(680, 1625)	0.1635	0.0407	57%	42.5%
	FDA007L	70	(16, 311)	2.0252	0.0565	323%	90	(18, 452)	2.3338	0.0659	371%	-13.3%
	FDA007M	878	(386, 2002)	0.5869	0.1447	135%	957	(830, 1103)	0.0000	0.0884	35%	87.9%
	FDA007N	840	(336, 2100)	0.7357	0.1381	155%	910	(652, 1270)	0.0881	0.0614	47%	82.9%
	FDA007O	206	(20, 2066)	4.8032	0.1409	824%	179	(23, 1375)	3.7563	0.1025	613%	22.0%
	FDA007P	1529	(693, 3374)	0.5468	0.1086	125%	1627	(1398, 1894)	0.0046	0.0785	33%	87.3%
	S21	4	(4, 4)	0.0000	0.0112	11%	4	(4, 4)	0.0000	0.0112	11%	0.0%
	S22	4	(4, 4)	0.0000	0.0112	11%	4	(4, 4)	0.0000	0.0112	11%	0.0%
S23	108	(65, 181)	0.2197	0.0940	75%	109	(69, 172)	0.1617	0.1091	68%	13.7%	
S22	4	(4, 4)	0.0000	0.0000	0%	4	(4, 4)	0.0000	0.0000	0%	NA	

Pn, pneumococcal serotype; **CI**, confidence interval; **CV**, coefficient of variation (expressed as a percent); **Lab**, variability among the laboratories; **Run(Lab)**, variability among the runs within each laboratory.

Supplementary Table S3. Effect of normalization on intra-laboratory variability. The reduction in variability within a laboratory due to normalization is shown. Estimates of various variance components and CVs from the ANOVA are also shown. Outliers are included in these analyses.

Serotype	Lab	Unadjusted			Normalized			Reduction in Variability (%)
		Variance Estimates		%CV	Variance Estimates		%CV	
		Run	SamplexRun		Run	SamplexRun		
Pn1	A	0.0010	0.0480	25%	0.0244	0.0556	33%	-39%
	B	0.0310	0.1052	45%	0.0000	0.1104	39%	19%
	C	0.0084	0.0381	24%	0.0298	0.0441	31%	-37%
	D	0.0122	0.0717	34%	0.0283	0.0548	33%	1%
	E	0.0010	0.0674	30%	0.0305	0.0753	38%	-35%
	F	0.0080	0.0480	27%	0.0077	0.0455	26%	5%
Pn3	A	0.0603	0.0640	42%	0.0166	0.0670	34%	33%
	B	0.0096	0.1415	47%	0.0315	0.1681	56%	-24%
	C	0.0144	0.0400	26%	0.0108	0.0378	25%	11%
	D	0.0047	0.0562	28%	0.0084	0.0304	22%	36%
	E	0.0133	0.0605	31%	0.0264	0.0648	35%	-19%
	F	0.0159	0.0478	29%	0.0144	0.0716	34%	-26%
Pn4	A	0.0315	0.1273	49%	0.0092	0.0937	38%	35%
	B	0.0120	0.0430	26%	0.0136	0.0763	35%	-39%
	C	0.0349	0.0329	30%	0.0073	0.0486	27%	18%
	D	0.0125	0.0500	28%	0.0080	0.0627	30%	-12%
	E	0.0052	0.0544	28%	0.0550	0.0490	38%	-43%
	F	0.0334	0.0723	38%	0.0000	0.0765	32%	28%
Pn5	A	0.0005	0.0458	24%	0.0067	0.0462	26%	-12%
	B	0.0195	0.0547	31%	0.0099	0.1102	41%	-38%
	C	0.0081	0.0239	20%	0.0045	0.0290	20%	-4%
	D	0.0121	0.0367	25%	0.0140	0.0351	25%	-1%
	E	0.0043	0.1091	40%	0.0466	0.1188	50%	-31%
	F	0.0106	0.0255	21%	0.0021	0.0229	17%	31%
Pn6A	A	0.0076	0.1377	46%	0.0071	0.0640	31%	51%
	B	0.0420	0.1331	52%	0.0000	0.2253	61%	-22%
	C	0.0074	0.0193	18%	0.0103	0.0312	23%	-36%
	D	0.0809	0.0862	50%	0.0068	0.0707	32%	54%
	E	0.0118	0.0964	39%	0.0555	0.0909	47%	-26%
	F	0.0320	0.0681	37%	0.0128	0.0717	34%	16%
Pn6B	A	0.0164	0.1053	42%	0.0439	0.1157	49%	-24%
	B	0.0483	0.0330	33%	0.0076	0.0464	26%	34%
	C	0.0041	0.0376	23%	0.0006	0.0356	21%	13%
	D	0.0301	0.0376	30%	0.0112	0.0709	33%	-18%
	E	0.0228	0.0550	32%	0.0593	0.0434	38%	-24%
	F	0.0248	0.0763	37%	0.0511	0.0698	42%	-16%
Pn7F	A	0.0057	0.1166	42%	0.0000	0.1210	42%	1%
	B	0.0488	0.0957	46%	0.0774	0.1864	67%	-45%
	C	0.0187	0.0258	23%	0.0006	0.0370	21%	15%
	D	0.0421	0.0573	37%	0.0119	0.0346	24%	53%
	E	0.0000	0.0594	28%	0.0378	0.0695	39%	-45%
	F	0.0088	0.1149	42%	0.0000	0.2052	57%	-40%
Pn9V	A	0.0000	0.1366	45%	0.0031	0.0955	37%	28%
	B	0.0532	0.1204	52%	0.0167	0.3011	76%	-45%
	C	0.0333	0.0384	31%	0.0170	0.0333	25%	30%
	D	0.1855	0.0510	63%	0.0211	0.0483	30%	71%
	E	0.0018	0.0516	26%	0.1302	0.0434	52%	-69%
	F	0.0341	0.0553	35%	0.0045	0.0640	30%	23%

Pn, pneumococcal serotype; **CV**, coefficient of variation (expressed as a percent); **Run**, variability among the runs within a laboratory; **SamplexRun**, variability associated with the interaction between test sample and assay run.

Supplementary Table S3 (continued). Effect of normalization on intra-laboratory variability. The reduction in variability within a laboratory due to normalization is shown. Estimates of various variance components and CVs from the ANOVA are also shown. Outliers are included in these analyses.

Serotype	Lab	Unadjusted			Normalized			Reduction in Variability (%)
		Variance Estimates		%CV	Variance Estimates		%CV	
		Run	SamplexRun		Run	SamplexRun		
Pn14	A	0.0200	0.0547	31%	0.0056	0.0498	27%	26%
	B	0.0014	0.0297	19%	0.0196	0.0318	25%	-39%
	C	0.0134	0.0250	22%	0.0000	0.0434	23%	-12%
	D	0.0125	0.0567	30%	0.0048	0.0481	26%	24%
	E	0.0265	0.0679	36%	0.0140	0.0774	35%	3%
	F	0.0977	0.0474	46%	0.0029	0.0533	27%	61%
Pn18C	A	0.0000	0.0956	36%	0.0005	0.0632	29%	33%
	B	0.0581	0.1697	61%	0.0591	0.2214	70%	-19%
	C	0.0111	0.0296	22%	0.0084	0.0333	23%	-2%
	D	0.1091	0.0722	53%	0.0206	0.0544	31%	59%
	E	0.0036	0.1550	49%	0.0474	0.1688	59%	-27%
	F	0.0549	0.0905	46%	0.0010	0.0800	33%	44%
Pn19A	A	0.0104	0.0646	31%	0.0074	0.0669	31%	1%
	B	0.0197	0.0408	28%	0.0000	0.0602	28%	0%
	C	0.0257	0.0269	26%	0.0066	0.0269	20%	36%
	D	0.0233	0.0572	33%	0.0005	0.0928	36%	-14%
	E	0.0000	0.0000	0%	0.0000	0.0000	0%	0%
	F	0.0618	0.0611	42%	0.0425	0.2222	67%	-54%
Pn19F	A	0.0186	0.0521	30%	0.0023	0.0388	22%	42%
	B	0.0578	0.1113	51%	0.0749	0.1651	63%	-30%
	C	0.0094	0.0313	22%	0.0065	0.0428	25%	-17%
	D	0.0463	0.0580	38%	0.0185	0.0469	29%	37%
	E	0.0027	0.1120	40%	0.0272	0.1917	60%	-48%
	F	0.0413	0.0481	35%	0.0021	0.0511	26%	41%
Pn23F	A	0.0111	0.1070	41%	0.0002	0.0913	35%	23%
	B	0.0486	0.0743	42%	0.0319	0.0825	40%	7%
	C	0.0172	0.0220	22%	0.0033	0.0179	16%	46%
	D	0.0614	0.0914	48%	0.0000	0.0799	33%	48%
	E	0.0370	0.1286	50%	0.0093	0.1006	39%	34%
	F	0.0015	0.0276	19%	0.0007	0.0349	21%	-18%

Pn, pneumococcal serotype; **CV**, coefficient of variation (expressed as a percent); **Run**, variability among the runs within a laboratory; **SamplexRun**, variability associated with the interaction between test sample and assay run.