**Bridging Human Pneumococcal Standard Reference serum 007sp to 89SF by using ELISA.**

*Streptococcus pneumoniae* Human Reference Serum, lot 89SF is used to measure antibody response in those vaccinated with any of several available pneumococcal vaccines. Serum 89SF is a serum pool from individuals vaccinated with a 23-valent pneumococcal polysaccharide vaccine collected in the late 1980s. The final volume of this collection was approximately 40 liters. It was agreed upon at the time by all parties that this would become the reference standard for all ELISAs measuring the concentration of antibodies reactive with the capsular polysaccharide of the pneumococcus. The aliquoted material is maintained and shipped from the Center for Biologics, Evaluation and Research/FDA. They ship approximately 150 vials of 89SF per year currently to laboratories all over the world. They have dispensed the final bulk of 89SF and are approaching limiting quantities for shipment.

In 2006, Dr. Milan S. Blake, Director, Division of Bacterial, Parasitic, and Allergenic Products, OVRR/CBER/FDA lead a program to expeditiously pursue a replacement for the Pneumococcal Human Reference Serum 89S. A protocol was written at CBER and a contract was funded jointly by CBER and DMID, NIAID. The protocol called for approximately 240 volunteers to be vaccinated with Pneumovax 23® having been previously examined and serologically tested to meet the inclusion criteria of the protocol. Once vaccinated, the volunteer would return at weeks 4 and 8 and donate one unit of blood that would be further processed into serum and stored at 2-8°C. The protocol was initiated in the spring of 2007 at the University of Iowa Medical School, Dr. Jack Stapleton being the principal investigator. Over 120 liters of human sera was collected of which 16 individual sera were researched for an addition standardization panel. The remaining sera were pooled, blended and fill/finish at a commercial CMO. The 6 ml lyophilized serum vials have been shipped to secure CBER storage facilities. Final vials have been further tested for adventitious agents, bioburden, moisture, and sterility. The plan is to make the new reference standard available for at least three types of assays: IgG ELISA, OPA, and multiplex immuno-assays.

On May 21, 2009, the FDA convened a meeting of the 007sp working group which included representatives from vaccine manufacturers, the Centers for Disease Control and Prevention, academic institutions, and the National Institute for Biological Standards and Control (NIBSC) (see appendix for working group members). At this meeting a basic plan was developed to initiate these bridging studies to link or bridge the replacement standard reference serum 007sp to the nearly exhausted 89SF. This is being done (1) because of the limiting supply of 89SF, (2) to maintain the standardization of assays, and (3) accelerate the use of these assays to hasten the development and deployment of pneumococcal conjugate vaccines.

The purpose of this document is to briefly describe the multi-laboratory study that successfully bridged 007sp to 89SF and assigned IgG antibody concentrations for 13 pneumococcal serotypes to 007sp. Additional studies are in progress to assay 12 well-characterized quality control sera using 007sp as the standard reference serum to measure

agreement with previously assigned values (using 89SF), and assaying 16 new quality control sera and assigning values to these for each of 13 serotypes. Results of these later phases will be published at the conclusion of the studies.

Five laboratories participated in the initial phase of the 007sp bridging exercise. Across all laboratories, 007sp was assayed at least 200 times for each serotype.

A random-effects analysis of variance (ANOVA) model was fitted for each of the 13 serotypes and used to estimate the assigned value and associated confidence interval (CI) for each serotype in 007sp. Data were analyzed after using a natural log transformation of ELISA IgG concentration. These values served as the outcome variable and included the overall mean as a fixed-effect (independent variable) and laboratories, plates and replicates as random-effects. Reproducibility was estimated for the labs and plates nested within labs. Repeatability was estimated using replicates within a plate and there were up to four replicates per plate.

Assigned values and CIs were estimated using the fitted ANOVA model by serotype. A 95% CI was estimated by serotype by accounting for the variance components between the labs, between plates within a lab and sample variability nested within plates and lab. Assigned values and associated 95% CIs were obtained by back-transforming the estimated log-transformed concentration and associated 95% CI.

**Participating Laboratories (in random order):**

University of Alabama, USA
Pfizer Vaccine Research, USA
GlaxoSmithKline Biologicals, Belgium
Institute of Child Health, University College London, UK
PPD Vaccines & Biologics Laboratory, USA on behalf of Merck Sharp & Dohme Corp

**007sp Assigned Values:**

| Type | IgG ELISA Concentration | Lower 95% CI | Upper 95% CI | N |
|---|---|---|---|---|
| 1 | 8.50 | 7.88 | 9.16 | 200 |
| 3 | 1.45 | 1.36 | 1.55 | 200 |
| 4 | 3.33 | 2.95 | 3.77 | 200 |
| 5 | 7.51 | 7.04 | 8.02 | 210 |
| 6A | 3.93 | 3.74 | 4.14 | 200 |
| 6B | 9.05 | 7.59 | 10.80 | 225 |
| 7F | 8.30 | 8.14 | 8.46 | 200 |
| 9V | 6.44 | 6.06 | 6.84 | 200 |
| 14 | 37.99 | 34.86 | 41.39 | 220 |
| 18C | 7.30 | 6.80 | 7.84 | 205 |
| 19A | 13.87 | 11.51 | 16.73 | 205 |
| 19F | 14.61 | 12.68 | 16.82 | 200 |
| 23F | 5.95 | 5.21 | 6.81 | 215 |

IgG ELISA concentrations were measured for a panel of 12 reference sera obtained from D. Goldblatt (UCL Institute of Child Health) and distributed by the National Institute for Biological Standards and Control (NIBSC; Potters Bar, Hertfordshire, United Kingdom), United Kingdom using both 89SF and 007sp as the reference standards.

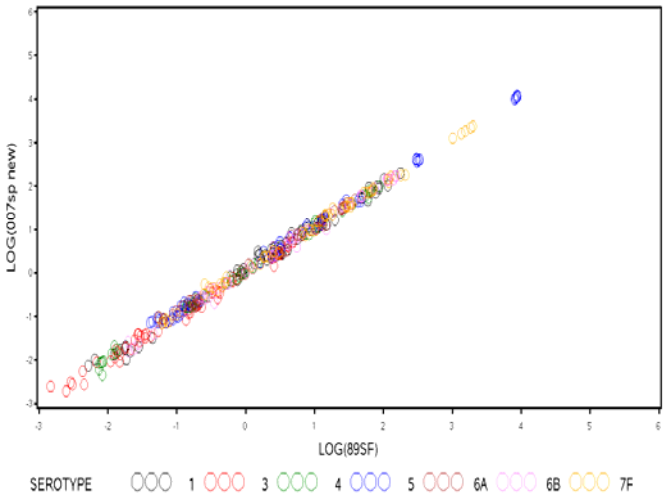
The next several pages contain scatterplots and graphs that illustrate the accuracy and precision of the estimated assigned values for 007sp compared to 89SF. The data were split into two pieces so reviewers may more easily inspect the results. The first section presents serotypes 1, 3, 4, 5, 6A, 6B, and 7F and the second section presents the remaining serotypes
9V, 14, 18C, 19A, 19F, and 23F.

The scatterplots show the high degree of agreement and correlation among the calculated concentrations using 007sp (vertical scale) vs 89SF (horizontal scale). A perfect level of agreement would yield a straight line with slope of 1 and intercept at 0. With rare exception, all data points cluster tightly about this line of identity.

The boxplots illustrate the deviation of the 007sp estimates from those obtained using 89SF. These plots offer more resolution than the scatterplots in that they relay more information regarding the deviation of the 007sp and 89SF estimates. In these plots, the box is defined by the 25th and 75$^{th}$ percentiles of the distribution; the horizontal line within the box represents the median or 50th percentile, and the asterisk signifies the mean. Vertical lines extend to the most extreme observation that is less than 1.5 times the interquartile range (75th to 25th percentiles), and the diamonds and boxes correspond to moderate and severe outlying assay values, respectively. Data above the dotted horizontal line indicates 007sp estimates are greater than estimates using 89SF. On the vertical axis, 2 indicates a point where the 007sp estimate was twice the 0089SF estimate. A value of ¼ indicates the 89SF estimate was four times the
007sp estimate. Boxes centered on the horizontal dotted line indicate a good agreement between the 007sp and 89SF estimates. By and large, the concentrations calculated by 007sp are within two folds (1/2 – 2.0) of those calculated using 89SF. Notable exceptions include serotypes 6B and 19A for lab 5 and serotype 23F for lab 4.

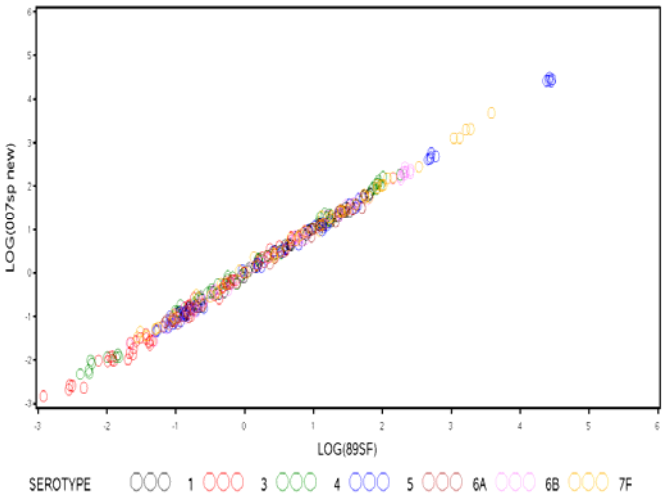
In general there is a high degree of agreement between the 007sp and 89SF estimates. This should inspire confidence in the validity of the 007sp estimates.

LAB 1



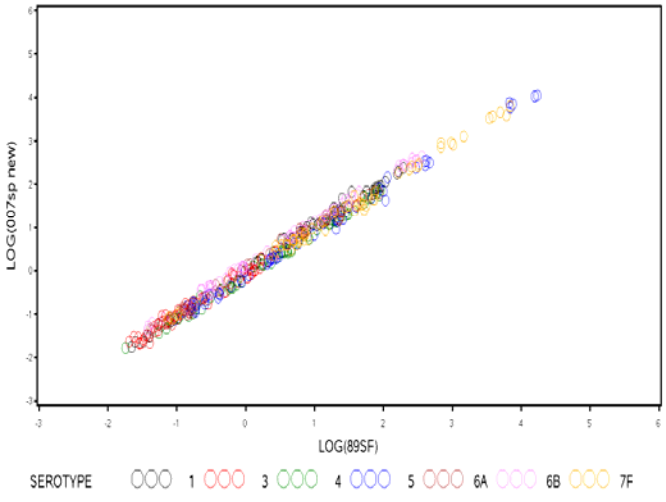

SEROTYPE 1 3 4 5 6A 6B 7F

LAB 2



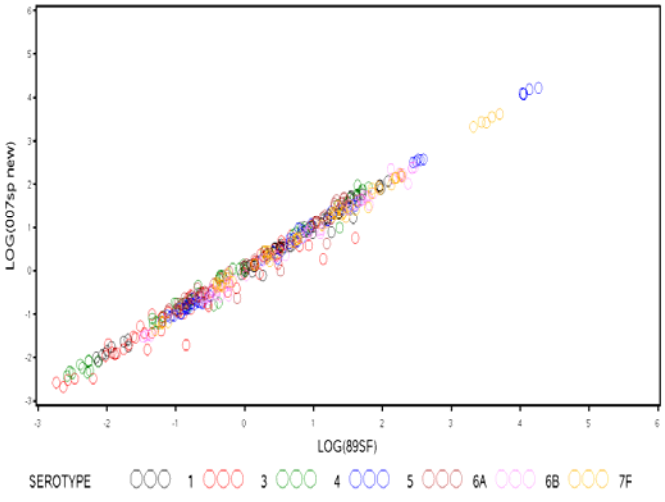

SEROTYPE 1 3 4 5 6A 6B 7F

LAB 3



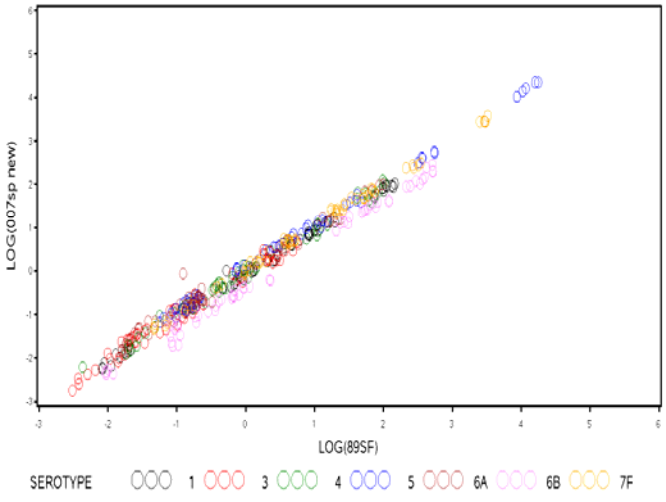

SEROTYPE 1 3 4 5 6A 6B 7F

LAB 4

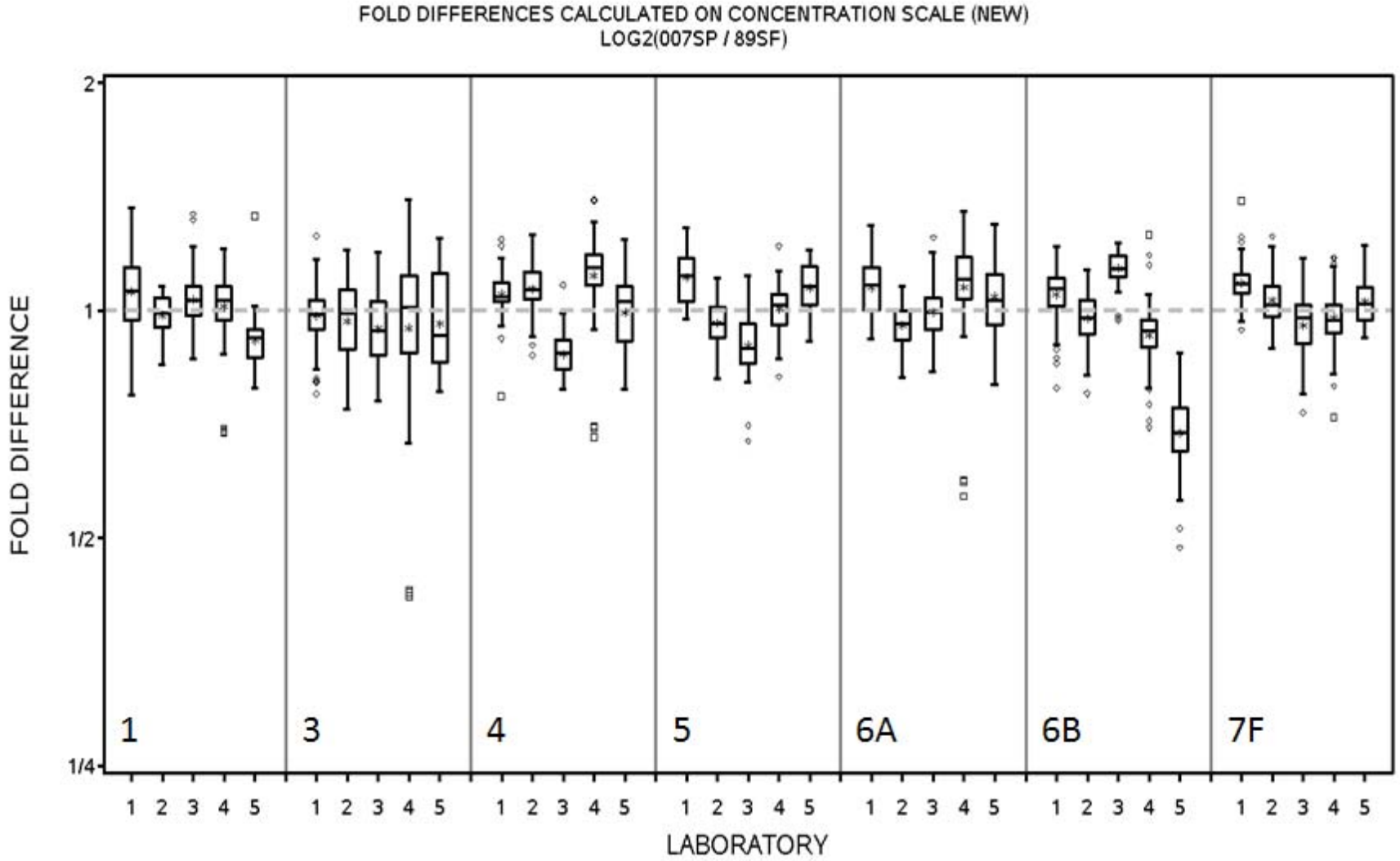



SEROTYPE 1 3 4 5 6A 6B 7F

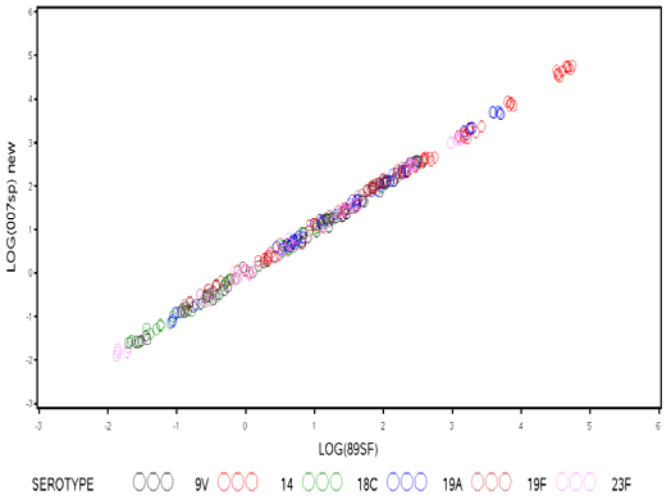
LAB 5



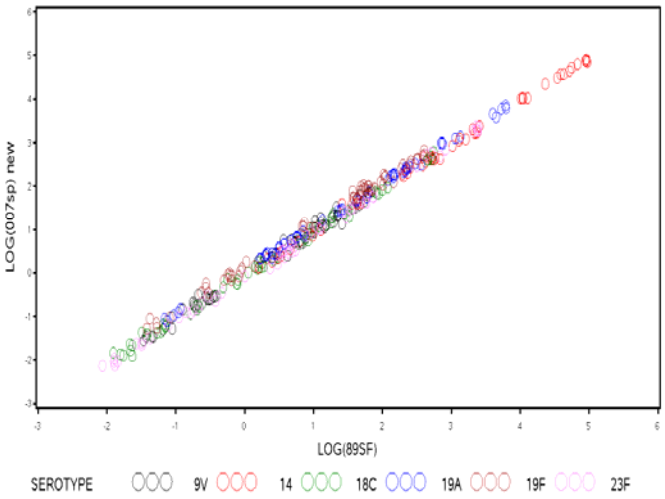

SEROTYPE 1 3 4 5 6A 6B 7F


FOLD DIFFERENCES CALCULATED ON CONCENTRATION SCALE (NEW)
LOG2(007SP / 89SF)
FOLD DIFFERENCE
2
1
1/2
1/4
1
3
4
5
6A
6B
7F
1 2 3 4 5
LABORATORY

LAB 1




LAB 2

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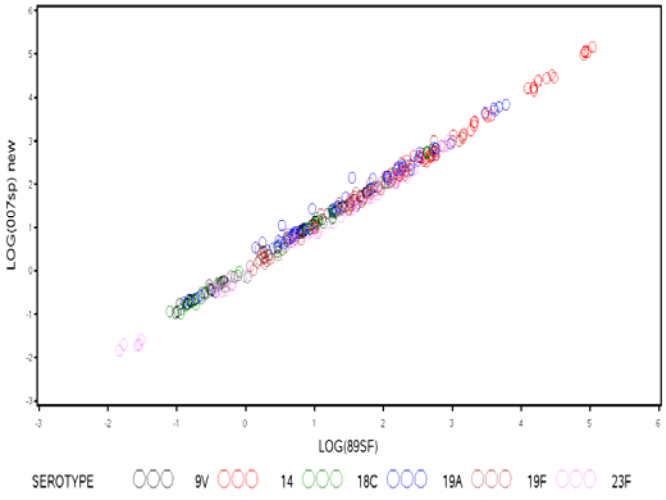
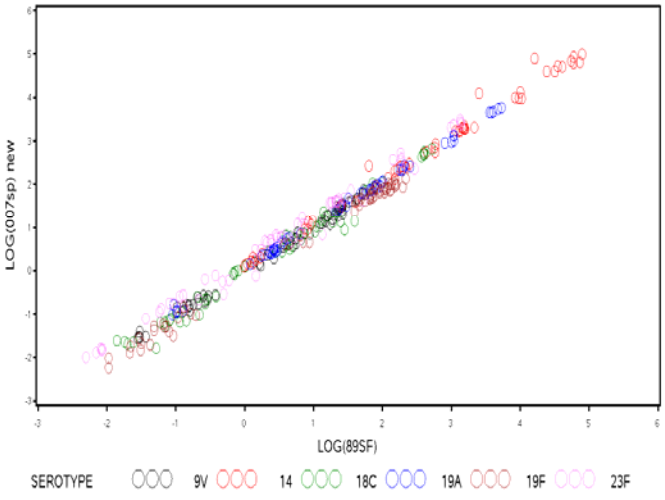
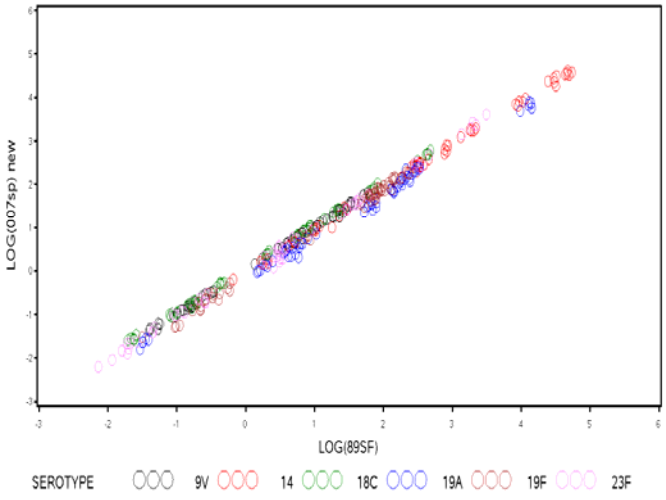
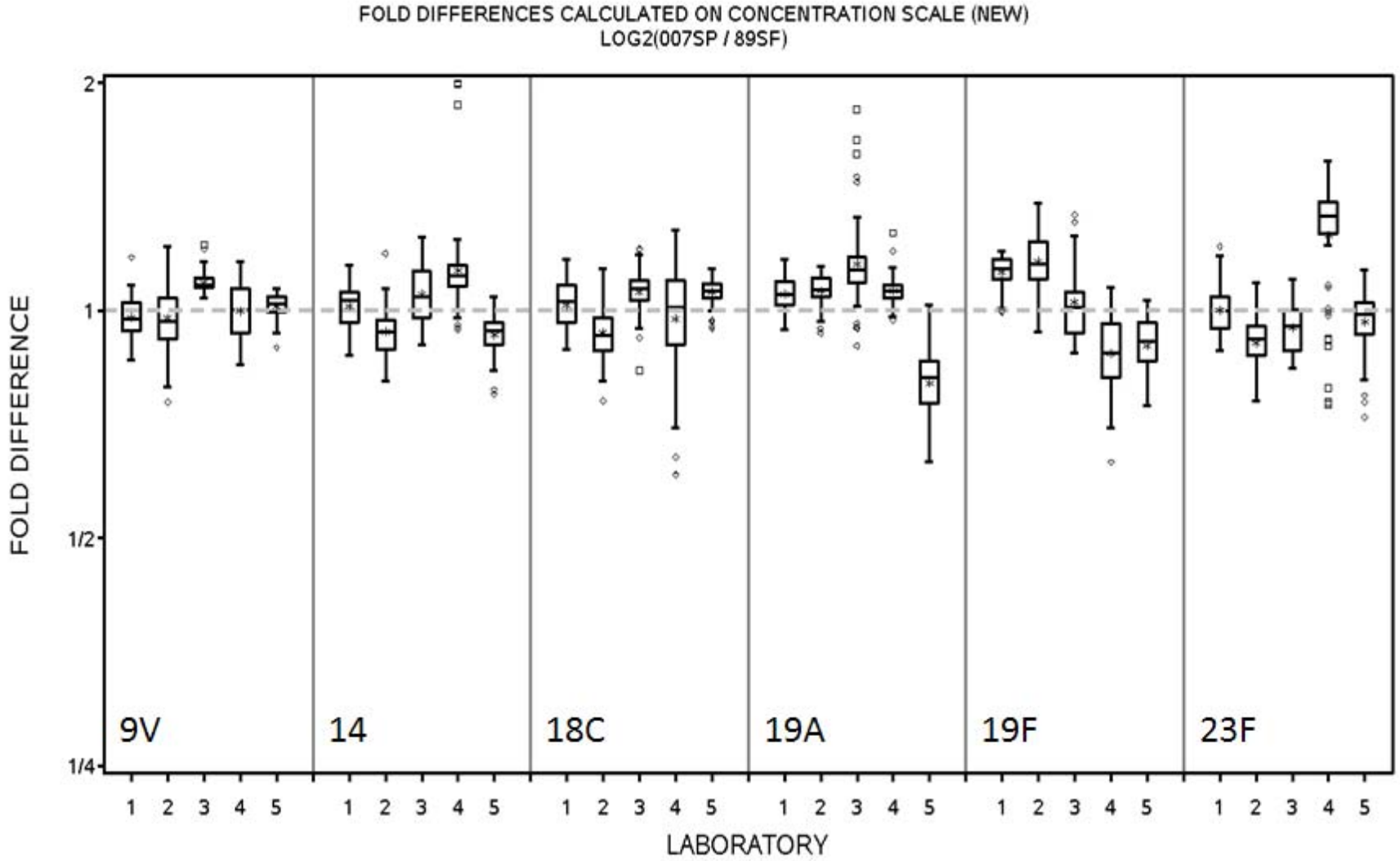



LAB 4




LAB 5




FOLD DIFFERENCES CALCULATED ON CONCENTRATION SCALE (NEW)
LOG2(007SP / 89SF)
FOLD DIFFERENCE
2
1
1/2
1/4
9V
14
18C
19A
19F
23F
1 2 3 4 5
LABORATORY

For further information please contact:
Brian Plikaytis [bdp1@cdc.gov], CDC, Atlanta
George Carlone [gmc3@cdc.gov], CDC, Atlanta
David Goldblatt [d.goldblatt@ucl.ac.uk], Institute of Child Health, University College London

**Appendix:** 007sp Working group membership:

FDA/CBER, USA: Milan Blake (Chair), Lucia Lee, Mustafa Akkoyunlu.
Center for Disease Control, USA: Brian Plikaytis, Charles Rose, George Carlone, Sandra Romero-Steiner, Daniel Schmidt.
University of Alabama, USA: Moon Nahm, Robert Burton.
Pfizer Vaccine Research, USA: Phil Fernsten, Peter Giardina, Kathrin Jansen.
GlaxoSmithKline Biologicals, Belgium: Nathalie Durant, Francoise Fievet.
National Institute for Biological Standards and Control, UK: Ian Feavers, Rory Care.
Institute of Child Health, University College London, UK: David Goldblatt, Lindsey Ashton.
PPD Vaccines & Biologics Laboratory, USA on behalf of Merck Sharp & Dohme Corp: Jennifer Raab, Lisa Kierstead.
Merck, West Point, PA, USA: Joseph Antonello.
PATH, Seattle, USA: Jeff Maisonneuve.