April 26, 2001 (updated 11/17/2009, 4/2/2012)

Pneumococcal ELISA standardization – Calibration Sera Panel A (for use with 89SF)

For Pneumococcal ELISA Calibration Sera Panel B (for use with 007sp) antibody levels, click here.

It is important that we understand the specificity and levels of antibodies required for protection against the different clinical forms of pneumococcal disease. As early as the 1940's it was clear that vaccine induced antibody in adults to the type specific capsular polysaccharides protected against invasive pneumococcal disease. The recent success of a seven valent pneumococcal conjugate vaccine confirms that antibodies to the capsular polysaccharide will also protect infants from bacteremic pneumococcal disease.

The only clear surrogate of protection against pneumococcal disease is the presence of opsonic antibodies. Therefore, any immune correlate of protective immunity needs to be correlated with induction of opsonic antibodies. ELISA methods that correlate well with induction of opsonic antibodies can be used to estimate protective antibody concentrations for prevention of invasive pneumococcal disease.

Different validated ELISA methods should measure similar concentrations of IgG anti-type specific polysaccharide antibodies. To assist in this goal, a set of calibration sera were assembled to assist labs in achieving comparability between laboratories.

With funding from WHO Dr. David Goldblatt obtained a set of 24 pairs of sera pre and post immunization from adults vaccinated with a 23-valent pneumococcal polysaccharide vaccine. These sera were analyzed in 12 labs using a common standardized assay protocol, and the study results appeared in J. Clin. Microbiol. (**38**:2043, 2000). Although not included in the cited publication, median antibody concentrations from the 12 labs for each serum against 10 serotypes were determined.

Subsequent to the above cited study it was found that the C polysaccharide did not fully remove non-type specific antibodies from sera from adults . Studies by Frasch et al. (Clin Diag Lab Immunol **8**:266, 2001) showed that further absorption by a heterologous pneumococcal polysaccharide (PS) improved the correlation between the measured antibody concentrations and opsonophagocytic titers. Although a number of different heterologous polysaccharides can be used, type 22F was chosen. Comparing 28 sera selected from the original 48 Goldblatt sera, selected for having lower assay to assay variability (CVs), the average fold decrease in antibody concentration following combined C PS and 22F PS absorption was 2.0 ± 0.6 for all types except type 14 for which 22F absorption had no effect (average fold difference was 0.9 ± 0.3 for the 28 sera for type 14).

A sub-set of 12 calibration sera were further selected from these 28 sera following studies done at CBER to identify those sera having the least cross-reactive antibodies removed by the 22F absorbent.

It was decided that different labs should assay these 12 sera using both the C and 22F PSs as absorbents, and that the 89SF reference would be absorbed with the C PS, but not with the 22F to retain the original antibody assignments in the reference. Studies by Wyeth-Lederle Vaccines indicated that on average the corrected 89SF values would be about 10% or less different from those now assigned for most types.

A meeting was held on October 16, 2000 in Geneva at the WHO headquarters to decide upon the antibody assignments for the 12 sera and the criteria by which laboratories, using the 12 sera, might evaluate their pneumococcal antibody assays. However, no agreement was reached at that time on these points. It was decided that for laboratories to achieve a target of at least 85% of determined antibody values within a percent error relative to the assigned value of 40% or less as originally proposed by the CDC group (J. Clin. Microbiol. (**38**:2043, 2000) would be difficult with only 12 calibration sera. The percent error is defined by the equation:

$$\frac{\left(\left|\text{assigned value} - \text{lab determined value}\right|\right)}{\text{assigned value}} \times 100$$

where | | indicates absolute value.

It was agreed at that meeting that, when evaluating pneumococcal IgG ELISA's, 9 of the 12 sera need to meet the 40% error test (see below). The summary report for the October 16 meeting is available from WHO, but recommendations from the report are as follows:

1.   There is consensus on use of a reference assay

- Based on performance criteria
  This is not prescriptive but a guidance for laboratories developing ELISA assays for Pneumococcal vaccines
- Use Wyeth Lederle Protocol as reference
- Use 4-5 parameter logistic regression analysis (see Plikaytis etal., J.Clin.Microb, 29:1439-1446, 1991)
- SSI CPs is available as a reference absorbant (WLV CPs also acceptable)
- Add 22F as additional absorbant from ATCC
- Use ATCC pneumococcal polysaccharide as coating antigen
- Medium-binding plates recommended
- Set up two reference laboratories to establish access and help for laboratories wanting to set up or trouble shoot their assays
    - WHO will have oversight of these laboratories to ensure consistency of results
    - These laboratories will become WHO collaborating centers
    - Establishment of the first Reference Laboratory at the Institute of Child Health in London has just been finalized with WHO (Contact details below)
    - Discussions around the establishment of a second Reference laboratory –at the NIH Pneumococcal Reference Laboratory in Rochester, NY are currently underway
    - Suggested laboratories- NIH Pneumococcal Reference Laboratory in Rochester, NY and Institute of Child Health in London
    - Funding will be sought through WHO
- Make the protocol available
- Quataert et.al, Clin. Diag. Lab. Immunol. 2:590-597, 1995

- www. vaccine.rochester.edu
- Produce guidance document

2. Assignment of values for calibration sera

- Provisional assigned values will be calculated on the basis of 12 sera using the median value of six participating labs
  - WHO will distribute the data to all participants
  - The data would be reviewed by the group of statisticians present at this meeting and results of discussions will be sent to WHO
- Investigate reasons for disparity of 6B values between laboratories
- Based on 12 sera analysed the group concluded that at least 75% of values for each serotype should have not more than 40% error relative to the assigned value (provisional)

Further analysis by CDC of the results from the six labs presenting data at the WHO meeting showed that the median and geometric means for individual serotypes were very similar. After further discussions between CBER and CDC, it was decided to provisionally assign the median values to the 12 sera for each of the seven types. Thus, provisionally assigned values are shown in Table 1. These seven types are the seven core types included in all pneumococcal conjugate vaccines now under evaluation. Provisional antibody assignments for types 1, 3, 5 and 7F will be added at a later date.

Table 2 shows for each serotype the number of determined antibody values that had percent errors of not more than 40% relative to the median values shown in Table 1. The data shown in Table 2 indicates that antibodies to all seven pneumococcal types could be measured with the agreed upon accuracy in most labs, and that, overall, no pneumococcal types presented greater difficulty in antibody measurements. For all types at least 3 labs achieved at least 11 of 12 sera (91.7%) with $\leq 40\%$ error, excepting types 14 and 19F where 2 of 5 labs had at least 11 sera with $\leq 40\%$ error. It is noted that antibody concentrations against type 14 were much higher than for the other types.

The overall conclusion is that the median values shown in Table 1 can be used to determine how well antibody measurements determined in different labs will agree.

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To obtain an aliquot of each of the 12 sera please contact (e-mail preferred)

Dr David Goldblatt   Email: d.goldblatt@ich.ucl.ac.uk
WHO Pneumococcal Reference Laboratory   Direct Tel +44 (0)207 813 8491
Institute of Child Health   Fax +44 (0)207 813 8494
30 Guilford Street
London, WC1N 1EH
United Kingdom

Sera are stored and will be distributed by the National Institute of Biological Standards and Control, Potters Bar, UK.

These sera are in very limited supply and should only be used for the purpose of calibration of Pneumococcal serotype specific IgG.

This document was drafted by Carl Frasch then reviewed and edited by Brian Plikaytis, George Carlone and David Goldblatt in 2001. Table 1 was updated in 2009 to replace two depleted sera.

File = D:\pneumo files\values for calibratn sera redacted.doc (4-26-01)
As of 4-26-2001 incorporates comments of all individuals listed above

TABLE 1 Provisional assignments of IgG antibody concentrations to pneumococcal types when the test sera are absorbed with both C and 22F polysaccharides *

Serum	Pneumococcal type						
	4	**6B**	**9V**	**14**	**18C**	**19F**	**23F**
728	3.1	0.9	2.8	10.7	2.5	9.4	7.0
730	7.2	4.9	1.5	66.1	3.2	9.7	1.4
738	2.3	12.9	7.7	18.4	6.1	2.5	1.6
742	6.2	9.9	2.1	7.4	7.4	11.5	0.9
744	1.8	23.3	10.0	3.7	18.1	9.5	2.6
752	9.7	10.2	17.0	27.8	9.9	64.1	13.2
754	14.6	3.3	15.8	160.8	4.9	14.1	7.9
756	8.5	7.7	7.5	57.2	8.0	15.8	5.6
760	2.3	2.1	1.8	19.2	3.3	6.8	2.4
764	4.0	23.2	8.3	17.2	6.4	21.7	18.1
768	0.7	2.5	4.6	14.0	1.5	3.5	1.4
770	2.0	8.3	4.2	115.6	2.8	7.3	11.6

* Provisional assignments based upon the median values reported from six
participating laboratories (CDC, CBER, WLV, KTL, ICH, and Roch)

TABLE 2 Performance of different labs* in their analysis of 12 calibration sera – Shown is the number of sera whose assayed value has a percent error ≤ 40%

Lab *	Type							
	4	**6B**	**9V**	**14**	**18C**	**19F**	**23F**	All types
A	12	12	10	12	11	12	11	80 (95%)
B	11	12	12	10	11	9	11	76 (90%)
C	9	6	11	12	11	9	12	70 (83%)
D	7	11	12	9	8	12	10	69 (82%)
E	11	7	9	8	11	7	9	62 (74%)
All labs	50 (83%)	48 (80%)	54 (90%)	51 (85%)	52 (87%)	49 (82%)	53 (88%)	------

* All labs were either government or biopharmaceutical labs experienced in
pneumococcal antibody analysis.