

IgA Calibrators LAPL-A-001

Developed for the determination of IgA anticardiolipin (aCL) antibodies by ELISA

For Research Use Only

Freeze-dried Calibrators

Enclosed are six freeze-dried serum samples. These samples will be referred as IgA *Calibrators*. The calibrators were selected to cover a wide range of predetermined concentrations of IgA aCL antibodies. They are suitable for the calibration of an ELISA test to detect IgA aCL antibodies in human serum. The test may be performed in a similar way to that described for the detection of IgG and IgM aCL antibodies (1).

Preparation and Storage of IgA Calibrators

Each Calibrator can be reconstituted by the addition of 120 μ l of sterile, distilled water (dH₂O). After the material has dissolved completely, it is recommended that aliquots (of say 15-20 μ l) be stored at -20 °C or lower for future use. When running the ELISA test, thaw an aliquot of each of the calibrators, dilute 1/50 in 10% adult bovine serum-PBS (10% ABS-PBS), then run each in duplicate using standard anticardiolipin ELISA test method (1).

Calibrators

The set included in this package contains six calibrators with different amounts of IgA aCL antibodies. The calibrators tested negative for IgG and IgM aCL antibodies. The calibrators were carefully selected to cover negative, low, medium and high positive values. APL units have been defined in our laboratory and assigned to each calibrator. One APL unit may be defined as the cardiolipin binding activity of approximately 1 μ g/ml of an affinity purified IgA aCL preparation from a high positive standard serum. (2)

Cut-Off Points and Levels of Positivity

We have determined the cut-off point to be 12-15 APL units. Values < 12-15 APL should be considered negative. However, it is recommended that every laboratory determine its own "cut-off" point in its own assay.

Preparing a Calibration Curve

The anticardiolipin assay is subject to considerable day to day variation. Reproducibility can be improved by constructing a calibration curve for every run. As determined for other anticardiolipin isotypes, we have found that a **log-logit plot** of optical density readings of calibration samples versus their APL values (Figure 1) gives the most reproducible and accurate results. (3)

The following method is recommended. Place the 6 IgA Calibrators in the first 6 duplicate wells (we often place these 6 samples in the last 6 duplicate wells of the plate, too, so that quadruplicate rather than duplicate measurements of each calibration standard can be made). Unknown serum samples are placed in subsequent wells. In the last step of the ELISA assay, the color reaction is stopped when the calibrator designated A4 achieves an O.D. reading of 0.9 ± 0.1 . A log-logit plot of O.D. readings of the 6 calibrators versus their values in APL units is then constructed (Figure 1). Several suitable software packages are available for computation of this curve and these can be performed using a computer attached to your ELISA reader. When a calibration curve is established, values of unknown samples can be derived from the calibration curve (in APL units), once their O.D. readings are known. Results are inaccurate for samples with IgA anticardiolipin levels exceeding 100 APL units. These samples will need to be diluted and rerun, and the values obtained multiplied by the dilution factor.

IgA Calibrators	
<i>Table of APL Units</i>	
Calibrator Designation	APL UNITS
A4	120
A6	80
A8	40
A9	20
A10	10
A13	2.7

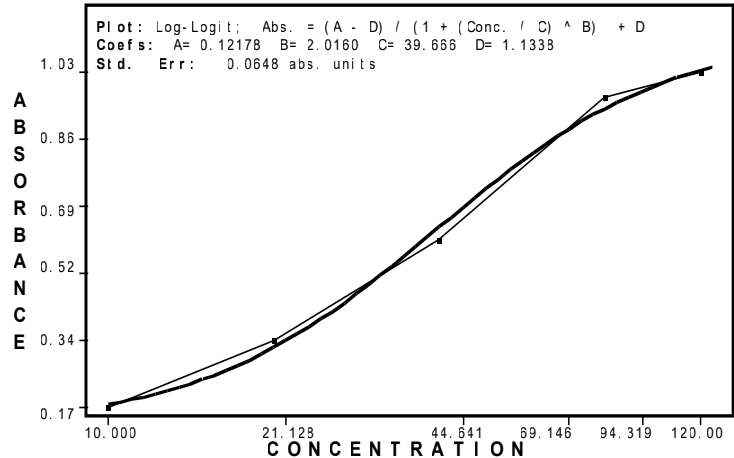


Figure 1: Plot of observance vs. concentration (APL units) using a log-logit fit. Concentrations of unknown samples can be derived from this curve, once the observances are known.

The binding of the IgA calibrators to *empty* and Phosphatidylcholine (PC) coated wells has been determined. The following table shows that the binding of the standards to PC and *empty wells* is insignificant.

<i>Binding Comparison of IgA Calibrators to CL, PC and EMPTY WELLS</i>			
Calibrator	Binding OD		
	CL	PC	Empty Wells
A4	0.899	0.290	0.315
A6	0.844	0.132	0.183
A8	0.588	0.134	0.143
A9	0.446	0.098	0.134
A10	0.329	0.132	0.123
A13	0.200	0.132	0.123

REFERENCES

- Harris, EN. (1990) Antiphospholipid Antibodies (Annotation). Brit. J. Haematol. 74: 1-9.
- Pierangeli S, Davis SA, Harris EN. (1992). IgA Calibrators for the Anticardiolipin Test Arthritis Rheum. 35, S359
- Pierangeli S, Greenberg R, Harris EN. (1989). Determination of the calibration equation which achieves best accuracy and precision of the anticardiolipin assay (abstract). Arthritis Rheum 32 (supplement), S123.

Guarantee - That we will replace any Product that does not perform as stated herein provided that the User reconstituted and stored the Standards or Calibrators in accordance with the enclosed instructions and an uncontaminated sample of the Product is returned to us for detailed analysis.