UTILITY OF INTERNATIONAL REFERENCE ANTIBODY CONTROLS IN STANDARDIZING IMMUNOASSAYS FOR ANTI-CARDIOLIPIN ANTIBODIES

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Abstract

Introduction: Detection of anti-cardiolipin antibodies (ACA) is important for anti-phospholipid syndrome. However the limitations of the existing immunoassays are that variable results are obtained on the same sample when tested on ELISAs from various suppliers. Attempts have been made to standardize these immunoassays with limited success. Two sets of reference standards are available i.e. Harris and Sapporo. The purpose of our study was to determine if the recently available Sapporo standard may provide better standardization for ACA assays.

Method: ACA positive (24) and negative (24) serum samples were tested on four IgM and four IgG commercial ACA ELISAs. ACA values were determined using the Harris and the Sapporo standards. Regression analyses was performed on each commercial ACA ELISA comparing Harris and Sapporo calibrators. In addition, clinical samples were assayed on different ACA ELISA's

Results: Variable results for cardiolipin antibodies of IgG and IgM were obtained on assays calibrated using Harris standards. In contrast, the Sapporo standard produced consistent values at all ranges of the calibration resulting in ACA values that are consistent. Anti-cardiolipin antibody values for clinical sera demonstrated less correlation when calibrated by the Harris Standard rather than the Sapporo Standard.

Comments: The use of Sapporo reference standard for calibration of ACA ELISA yields greater correlation of values among different manufacturer's assays as compared to the commonly used reference standard. Regression analysis demonstrates the superior performance of the Sapporo standard.

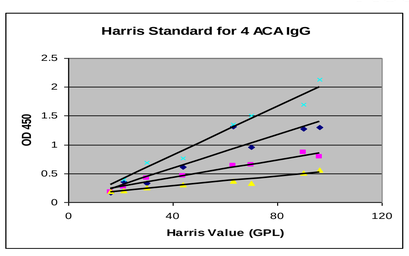
Introduction

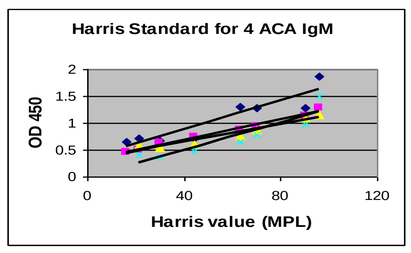
Since the development of an ACA ELISA in 1985, there have been numerous efforts to standardize the results for comparison among different ACA ELISA manufacturers. Advances have been made in plate coating procedures and material inputs (i.e. hu-β2GP1), despite this the standardization of ACA has been elusive. Currently, most ACA ELISA are calibrated to the Harris Standard, a polyclonal sera (IgM or IgG) that is calibrated to the previous generation of positive sera. In 1994 new monoclonal standards were developed. While these monoclonal standards do not mimic the heterogeneity of the polyclonal standards, their greater stability and wider availability make them excellent candidates for standardization. Most commercially available ACA maintain primary calibration to the Harris Standard, with only a few ACA allowing for secondary calibration to the Sapporo Standard. Here we advocate the use of the Sapporo Standard as the primary reference material for ACA IgG and ACA IgM ELISA based upon the greater correlation between commercially available ACA.

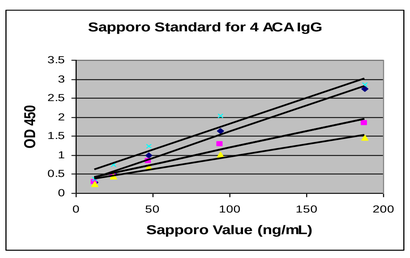
Materials and Methods

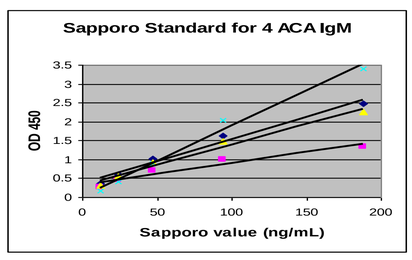
For the purpose of this study 4 commercially available ACA IgM and ACA IgG ELISA including two (ACA IgG and ACA IgM) produced by IMMCO Diagnostics were used. The Harris and Sapporo Standards were assayed on these ACA ELISA alongside 10 normal sera, 14 non-APS autoimmune disease control sera and 24 APS positive sera. Harris values used are 96, 90, 70, 65, 47, 30, 21, 16 GPL and 96, 88, 60, 47, 39, 33, 24, 13 MPL. Sapporo Standard values are 188,96,48,24, and 12 ng/mL for both IgM and IgG ACA assays. The sera samples were assayed within two days by the same individual. Regression analysis was performed on the values obtained by the Harris and Sapporo Standards on the 4 ACA IgG and ACA IgM ELISA. Regression analysis was also performed on the values of the 48 clinical sera obtained through a calibration curve plotted from the Harris Standard or the Sapporo Standard.

Results

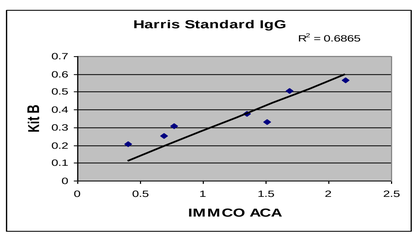


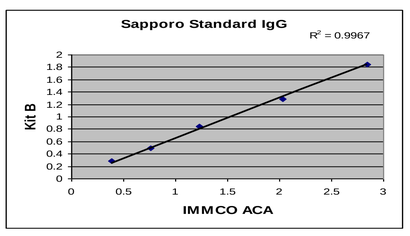






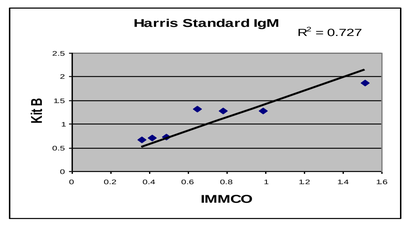
Comparison of Harris Standard and Sapporo Standard on multiple commercially available ELISAs. The Harris and Sapporo Standards were assayed on 4 ACA IgG and 4 IgM ACA ELISA ELISAs. Harris results were plotted as OD450 versus Harris value expressed as MPL (top right graph) or GPL (top left graph). Sapporo results were plotted as OD450 versus Sapporo value IgM (bottom right graph) and IgG (bottom left graph) expressed in ng/mL.

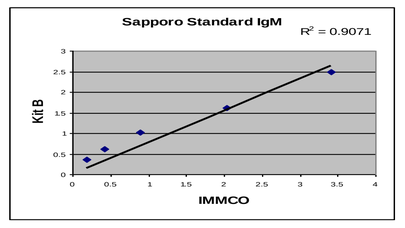




R^2 values of Standards for ACA IgG						
ELISA	IMMCO		C		B	
A	Harris	0.8299	Harris	0.4912	Harris	0.8375
	Sapporo	**0.9912**	**Sapporo**	**0.9291**	**Sapporo**	**0.9963**
B	Harris	0.8448	Harris	0.7525		
	Sapporo	**0.9969**	**Sapporo**	**0.9571**		
C	Harris	0.8097				
	Sapporo	**0.9641**				

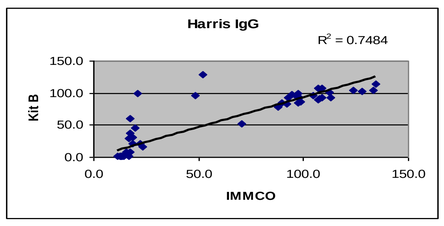
Regression analysis for Harris Standard and Sapporo Standard between ACA IgG ELISAs. Comparison of Harris Standard value (OD450) on commercially available ACA ELISA B versus IMMCO ACA ELISA (right graph). Comparison of Sapporo Standard value (OD450) on commercially available ACA ELISA B versus IMMCO ACA ELISA (left graph). The Sapporo Standard provides a greater correlation between ELISA B ACA IgG and IMMCO ACA IgG. Table of R-squared values of 4 ACA IgG ELISA by Harris Standard (black text) and by Sapporo Standard (red text). For all ACA IgG comparisons the use of the Sapporo Standard as the surrogate calibrator demonstrates a higher degree of correlation between commercially available ELISAs.

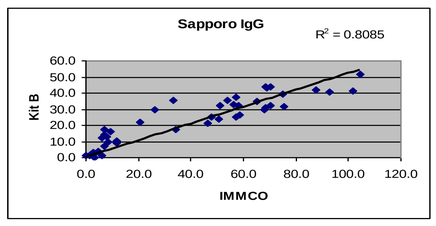




R^2 values of Standards for ACA IgM						
ELISA	Immco		C		B	
A	Harris	0.7709	Harris	0.9266	Harris	0.7537
	Sapporo	**0.8824**	**Sapporo**	**0.9609**	**Sapporo**	**0.9493**
B	Harris	0.8427	Harris	0.6761		
	Sapporo	**0.9391**	**Sapporo**	**0.9998**		
C	Harris	0.7576				
	Sapporo	**0.9425**				

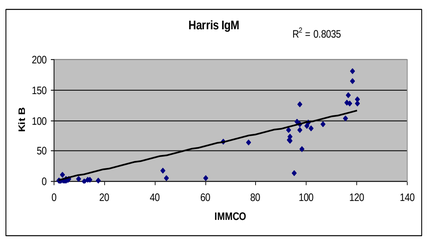
Regression analysis for Harris Standard and Sapporo Standard between ACA IgM ELISAs. Comparison of Harris Standard value (OD450) on commercially available ACA IgM ELISA B versus IMMCO ACA IgM ELISA (right graph). Comparison of Sapporo Standard value (OD450) on commercially available ACA IgM ELISA B versus IMMCO ACA IgM ELISA (left graph). The Sapporo Standard provides a closer correlation between ELISA B ACA IgM and IMMCO ACA IgM. Table of R-squared values of 4 ACA IgM ELISA by Harris Standard (black text) and by Sapporo Standard (red text). For all ACA IgM comparisons the use of the Sapporo Standard as the surrogate calibrator demonstrates a higher degree of correlation between commercially available ELISAs.

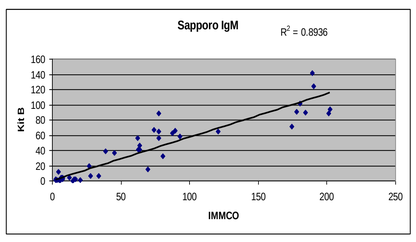




ACA IgG regression analysis for clinical sera						
ELISA	Immco		C		B	
A	Harris	0.8189	Harris	0.5492	Harris	0.8549
	Sapporo	**0.9032**	**Sapporo**	**0.8731**	**Sapporo**	**0.9031**
B	Harris	0.7484	Harris	0.5961		
	Sapporo	**0.8085**	**Sapporo**	**0.8293**		
C	Harris	0.7924				
	Sapporo	**0.8610**				

Regression analysis for Clinical sera between ACA IgG ELISA based on Harris Standard or Sapporo Standard. Comparison of Harris Standard value (MPL) on commercially available ACA IgG ELISA B versus IMMCO ACA IgG ELISA (right graph). Comparison of Sapporo Standard value (ng/mL) on commercially available ACA IgG ELISA B versus IMMCO ACA IgG ELISA (left graph). The Sapporo Standard provides a closer correlation of clinical sera value between ELISA B ACA IgG and IMMCO ACA IgG. Table of R-squared values of 48 clinical sera in a direct comparison of 4 ACA IgG ELISA by Harris Standard (black text) and by Sapporo Standard (red text).





ACA IgM regression analysis for clinical sera						
ELISA	Immco		C		B	
A	Harris	0.7709	Harris	0.9266	Harris	0.7537
	Sapporo	**0.8824**	**Sapporo**	**0.9609**	**Sapporo**	**0.9493**
B	Harris	0.8035	Harris	0.6761		
	Sapporo	**0.8936**	**Sapporo**	**0.9998**		
C	Harris	0.7576				
	Sapporo	**0.9425**				

Regression analysis for clinical seras between ACA IgM ELISAs based on Harris Standard or Sapporo Standard. Comparison of Harris Standard value (MPL) on commercially available ACA IgM ELISA B versus IMMCO ACA IgM ELISA (right graph). Comparison of Sapporo Standard value (ng/mL) on commercially available ACA IgM ELISA B versus IMMCO ACA IgM ELISA (left graph). The Sapporo Standard provides a closer correlation of clinical sera value between ELISA B ACA IgM and IMMCO ACA IgM. Table of R-squared values of 48 clinical sera in one to one comparison of 4 ACA IgM ELISA's by Harris Standard (black text) and by Sapporo Standard (red text).

Conclusions

1) The Sapporo Standard yields greater correlation of clinical sera among different ACA ELISA's
2) The Sapporo Standard demonstrates superior performance by regression analysis.
3) Use of Sapporo Standard Calibrated ACA ELISA would allow for better correlation between different ELISA manufacturers.

References

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