Louisville APL DIAGNOSTICS

REF	LAPL-GM-300
REF	LAPL-GM-300G
REF	LAPL-GM-300M

Calibrators for the Measurement of Anticardiolipin Antibodies IgG and IgM

For Research Use Only (USA)

IVD **For *In Vitro* Diagnostic Use**

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INSTRUCTIONS MANUAL

Calibrators LAPL-GM-300
Calibrators LAPL-GM-300G
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1 – INTENDED USE

The set of Calibrators LAPL-GM-300 has been designed for the construction of calibration curves in immunoassays for the detection of IgG and IgM anticardiolipin antibodies (aCL). These calibrators may be used to validate and/ or to verify performance of aCL assays.

2 – EXPLANATION OF THE PRODUCT

Enclosed are freeze-dried serum sample(s). These samples will be referred to as *Calibrators*

The Calibrators may be used in the construction of a calibration curve.

The Calibrators are suitable for the calibration of ELISA or other immunoassays to detect IgG and IgM aCL antibodies in human serum or plasma.

Calibrators labeled as GI, GII, GIII, GIV, GV, GVI, and GVII (blue labels) should be used in the determination of IgG aCL antibodies.

Calibrators labeled MI, MII, MIII, MIV, MV, MVI, and MVII (yellow labels) should be used for the determination of IgM aCL antibodies.

If you have not yet set up an assay, we suggest you read references 1-7.

This product replaces the product LAPL-GM-200 (set of 8 calibrators for IgG and IgM aCL, distributed between 2001 and 2012), and other similar products named LAPL GM-100 (set of 8 calibrators for IgG and IgM aCL, distributed between 1997 and 2001), LAPL-GM-001 distributed between 1990 and 1997 and the “original Harris” standards (named I, II, III, IV and V), distributed before 1990.

3 – DETERMINATION OF THE VALUE OF THE IgG/IgM CALIBRATORS

These new sets of Calibrators have been prepared by mixing various proportions of several positive sera for aCL from patients diagnosed with the Antiphospholipid Syndrome (APS) (8-10) and negative sera for aCL from healthy control donors. The calibrators have been prepared in a manner that covers a wide range of concentrations of IgG and IgM aCL (11). .

The value assigned to each calibrator was obtained after testing the blending of sera 45 times in a FDA cleared aCL ELISA. These values in GPL (Table 1) and MPL (Table 2) units should be used to construct the calibration curve in your assay.

In each calibrator, IgG and IgM aCL levels are reported in GPL and MPL units, respectively.

GPL: One GPL unit is defined as the cardiolipin binding activity of 1 μg/ml of an affinity purified IgG aCL preparation from a standard serum (REY) (12,13).

MPL: One MPL unit is defined as the cardiolipin binding activity of 1 μg/ml of an affinity purified IgM aCL preparation from a standard serum (DUF).

Table 1: Value **of IgG Calibrators in GPL**

Calibrator	mean Value in GPL
GI	120
GII	90
GIII	70
GIV	45
GV	30
GVI	10
GVII	5

Table 2: Value of IgM Calibrators in MPL

Calibrator	mean Value in MPL
MI	80
MII	70
MIII	60
MIV	45
MV	30
MVI	10
MVII	5

4 – PERFORMANCE DATA REPORT

4.1 – Comparison of the Calibrators LAPL-GM-300 against LAPL-GM200 and the "original Harris" calibrators.

The LAPL-GM-300 Calibrators were tested in triplicate on three consecutive runs using the "Original Harris" or LAPL-GM-200 calibrators to construct the calibration curves and the mean values were determined and the Spearman correlation coefficients were obtained between the "expected" values and the "observed" values (Table 3). Recovery was also calculated (Tables 4a and 4b)

Table 3: Spearman correlation of LAPL GM-300 tested using "Original Harris" or LAPL-GM-200 as calibrators

Calibration curve used	IgG GM-300 Corr Coef (R^2)	IgM GM-300 Corr Coef(R^2)
Original Harris	0.99	0.98
LAPL-GM-200	0.99	1.00

Table 4a: Recovery of the LAPL-GM-300 calibrators when tested in the aCL assay using "Original Harris" to construct the calibration curve

Standard	Expected Value	Observed Value	Recovery %
GI	120	110.0	92
GII	90	89.0	99
GIII	70	65.3	93
GIV	45	42.7	95
GV	30	34.5	115
GVI	10	12.3	123
GVII	5	4.0	
MI	80	87.9	109
MII	70	67.9	97
MIII	60	55,6	93
MIV	45	41.0	91
MV	30	28.0	93
MVI	10	10.7	107
MVII	5	6.0	120

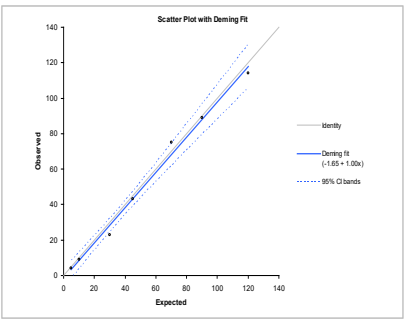
Table 4b: Recovery of the LAPL-GM-300 calibrators when tested in the aCL assay using LAPL-GM-200 to construct the calibration curve

Standard	Expected Value	Observed Value	Recovery %
GI	120	114.0	95
GII	90	89.0	99
GIII	70	75.0	107
GIV	45	43.0	96
GV	30	23.0	110
GVI	10	9.0	90
GVII	5	4.0	80
MI	80	76.5	96
MII	70	65.0	93
MIII	60	53.0	88
MIV	45	40.0	89
MV	30	25.0	83
MVI	10	9.5	95
MVII	5	3.0	60

4.2 – Linearity: Linearity of the LAPL-GM-300 calibrators was evaluated by testing the 7 IgG and 7 IgM calibrators in triplicates, in three different occasions in the aCL ELISA using either the "Original Harris" calibrators or LAPL-GM-200 calibrators to construct the calibration curve. Linearity was evaluated to determine if the calibrators' values follow a linear dilution pattern in the aCL assay, using Analyze-it-for Excel software. As allowable non-linearity, the use of 15% and a unit value that corresponds 20% of the assay's cut-off was used (14). Deming regression was performed and a R^2 value of ≥ 0.95 in all cases. (Figures 1 and 2)

Figure 1: Linearity Deming fit plots for IgG (a) and IgM (b) GM-300 calibrators when using "Original Harris" to construct the calibration curve)

1a) IgG



1b) IgM

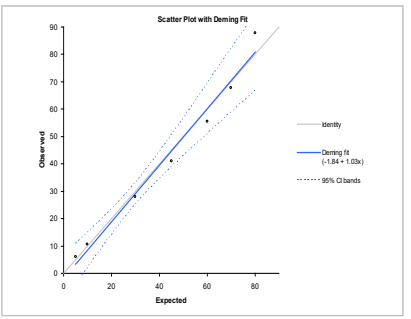
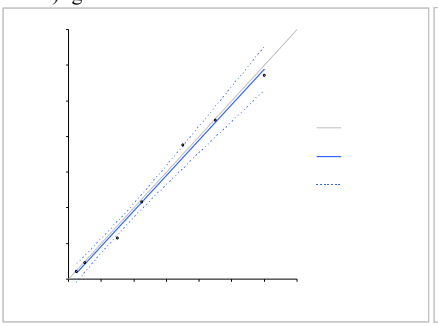
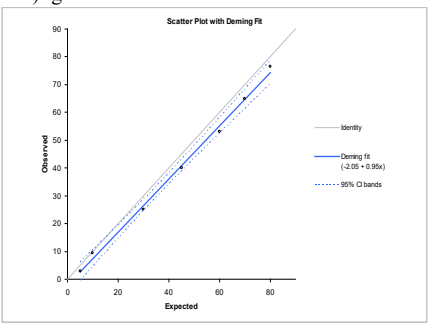


Figure 2: Linearity Deming fit plots for IgG (a) and IgM (b) GM-300 calibrators when using GM-200 calibrators to construct the calibration curve.

1a) IgG



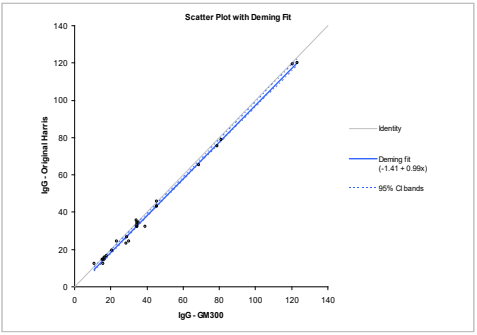
1b) IgM



4.3 – Evaluation and Comparison of Patient Sera in aCL assays using the GM-300 calibrators, and the GM-200 or the Original "Harris" Calibrators.

Twenty-five patient sera with aCL antibodies titers at different levels of positivity for IgG and IgM aCL antibodies, and two of the International "KAPS" standards were evaluated in the aCL ELISA using GM-300 calibrators to construct the calibration curve. The same samples were tested in aCL assays using either the GM-200 calibrators or the "Original Harris" calibrators to construct the calibration curve. Bland and Altman (difference plots) (15) and Deming regression analyses were conducted. In all cases, a statistically significant bias was not detected (15% allowable difference) for all the possible combinations. All the patient samples were within 95% confidence interval (CI). One example is shown in Figure 3.

Figure 3: Example of Method Comparison Deming Fit for GM-300 and "Original Harris" calibrators IgG



4.4 – Commutability Studies.

Twenty-seven patient sera were evaluated in two commercial kits (Kit A) and (Kit B). In the same assay the GM-300 calibrators were run. Deming Regression analysis was performed and the 95% CI for the regression line was calculated. In order to determine commutability of the GM-300 standards, the values obtained for the standards were plotted in the same graphs (16). GM-300 were found to be commutable in the two aCL assays, since all 7 IgG and 7 IgM calibrators fell within the 95% CI. (Figure 4).

Figure 4. Commutability plots for the IgG GM-300 calibrators (a) and for the IgM GM 300 calibrators (b).

Fig 4 (a) IgG

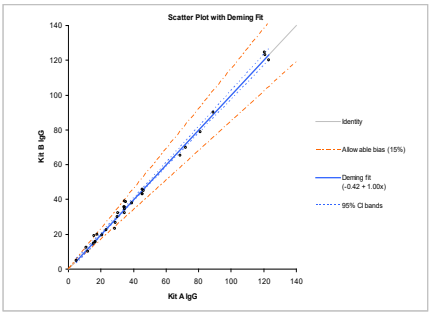
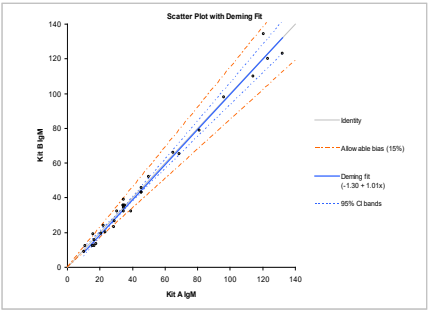


Fig 4 (b) IgM



4.5 – Traceability

The standards LAPL-GM-300 are traceable in GPL and MPL units to the "Original Harris" calibrators for aCL test (named: I, II, III, IV and V), prepared in 1986 (see validation and comparison data in previous paragraphs) (12,13). GPL and MPL units were defined as the phospholipid binding activity of one µg/ml of affinity purified IgG aCL (for GPL) or IgM aCL (for MPL) antibodies.

4.6 – Binding to other phospholipids and to $\beta_2$glycoprotein I.

The LAPL-GM-200 Calibrators have been standardized using cardiolipin as antigen in ELISA plates. Binding to other negatively charged phospholipids and $\beta_2$glycoprotein I may occur. However, no units of measurement were established for other phospholipids and/or $\beta_2$glycoprotein I for these calibrators.
In addition, the binding of these Calibrators to "empty" or non-coated plates and to phosphatidylcholine has been determined using an aCL ELISA assay. The binding to "empty" wells as well as to wells coated with 50 µg/ml phosphatidylcholine (PC) was negligible.

5 – SAMPLE PREPARATION AND STORAGE OF IgG/IgM CALIBRATORS

Each Calibrator should be reconstituted with 250 µl of sterile, distilled water. Allow the reconstituted material stand for 15 minutes; swirl gently to dissolve any remaining un-dissolved material. After the material has dissolved completely, divide each sample in aliquots (for example 10-20 µl). Aliquots of each sample should be stored at temperatures ≤ -20ºC for future use.

Do not freeze and thaw the reconstituted material repeatedly

6 – COMPONENTS

6.1 - Content of the set of 14 Calibrators LAPL-GM-300

The content is divided in two separate sets of:

7 Calibrators for IgG (LAPL-GM-300G)

7 Calibrators for IgM (LAPL-GM-300M)

Inspect the content against the list below

LAPL-GM-300G

7 - Vials with lyophilized sera identified with blue labels: GI, GII, GIII, GIV, GV, GVI, GVII, each vial should be reconstituted with 250 µl of sterile dH_2O. *

LAPL-GM-300M

7 - Vials with lyophilized sera identified with yellow labels: MI, MII, MIII, MIV, MV, MVI, MVII, each vial should be reconstituted with 250 µl of sterile dH_2O. *

*** Contains Sodium Azide 0.2% as preservative.**

6.2 Warnings

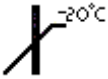
- This product should only be used by appropriately trained personnel.
- Materials of human origin included in the **LAPL-GM-300 Calibrators** have tested negative for HIV-I antibodies and Hepatitis B surface antigen. However, these materials and other sera to be tested should be handled as if they were infectious.
- Sodium Azide under acidic conditions yields hydrazoic acid, a very toxic compound. Azide compounds have been classified, under the directives of the European Community (CEE) as **Xn (Harmful)** and should be discarded with running water to avoid deposit in the piping system
- Avoid contact with skin or mucous membranes of calibrators or samples. If an accident occurs, rinse the area affected immediately with water and consult a physician

R20 Harmful if inhaled.
R21 Avoid contact with skin.
R22 Harmful if swallowed.
R32 Contact with acids liberates very toxic gas.
S2 Keep out of the reach of children.
S13 Keep away from food, drink, and animal feeding stuffs.
S36 Wear suitable protective clothing.
S37 Use gloves.
S46 If swallowed, seek medical advise immediately and show this container or label.

6.3 Required materials but not provided

- Micropipet / Multichannel pipette to deliver 5-1000 µl
- Vials for storage in the freezer
- Distilled water.
- Vortex mixer

6.4 Storage and Stability



It is recommend that the ***unopened - not reconstituted***, LAPL-GM-300 Calibrators be stored at temperature ≤ -20ºC for up to three years from the date of acquisition.
After reconstitution, it is recommended to store the aliquots at ≤ -20ºC for up to two years from the date of reconstitution.

The aliquots in use can be stored in the refrigerator at 2-8ºC for up to 7 days.

- Do not use the calibrators beyond the expiration date.
- **Do not freeze and thaw** the reconstituted material.

7 – INSTRUCTIONS TO USE THE CALIBRATORS.

7.1 Procedural Precautions

- Read instruction booklet in its entirety and review prior to testing.
- Bring the calibrators to room temperature before use.
- Use clean tips for each calibrator.

7.2 Elaboration of the calibration curve using the LAPL-GM-300 Calibrators

- A calibration curve should be constructed every time for each isotype (IgG or IgM) using only:
  - The set of 7 Calibrators for IgG (LAPL-GM-300G)
  - The set of 7 Calibrators for IgM (LAPL-GM-300M).
- A calibration curve must be prepared for each assay.
- It is recommended to use a "log-log" or "log-logit" plot. It has been shown that using either one of those plots, a more reproducible result can be obtained (17).
- It is recommended that the standard curve be run in duplicate wells.
- If the standard aCL ELISA assay is used, use protocol as described elsewhere (1-7)
  - After stopping the color reaction the plate should be read.
  - Plot mean O.D. readings and construct the curve of the seven Calibrators versus their values in GPL units (Table 1) or MPL units (Table 2).
  - The best suitable types of curve are log-log (Figure 5) and log-logit (Figure 6).
  - It is recommended to use a computer with validated software. Several suitable software packages are available for construction of the curve and extrapolation of the results in GPL or MPL
  - Results in excess of 120 GPL or 80 MPL (above the analytical measuring range of the assay for IgG and IgM, respectively) tend to be inaccurate; therefore, samples with values over those values should be diluted, retested and the value multiplied by the dilution factor.

8 – EXAMPLE OF A CALIBRATION CURVE

Figure 5. Example of a calibration curve for IgG aCL antibodies using the LAPL-GM-300 Calibrators in a log-log plot

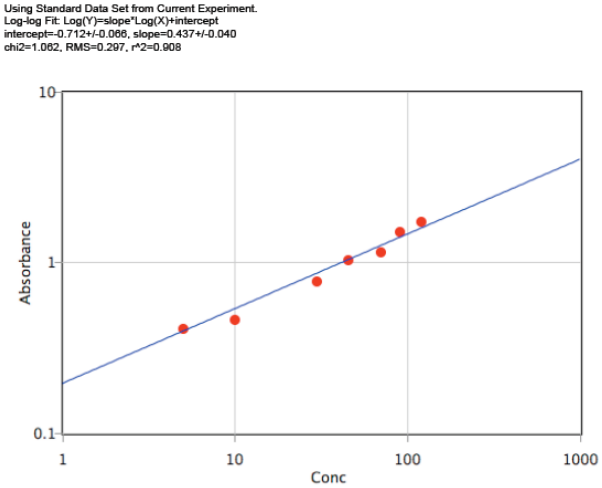
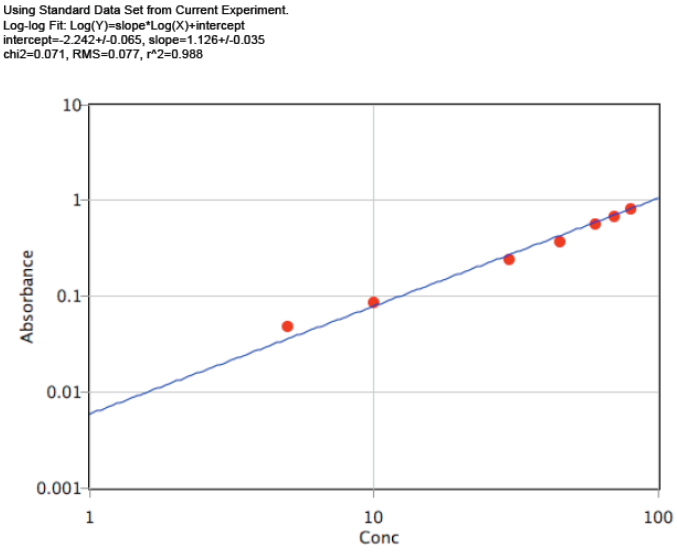


Figure 6. Example of a calibration curve for IgM aCL antibodies using the LAPL-GM-300 Calibrators in a log-log plot



9 – DEGREE OF POSITIVITY

- In general, it is accepted that most patients with repeated thrombosis or fetal loss in APS have the IgG isotype, with levels above 40 GPL units (medium and high positive) (18-20).
- Although less frequent, APS patients may also have IgM aCL antibodies, in addition to IgG or even only IgM aCL antibodies.
- The results should be reported as HIGH, MEDIUM or LOW positive or negative, (Table 5), in addition to GPL or MPL units.
- Better inter-laboratory agreement may be achieved by reporting results by ranges of positivity (12,13).

Table 5. Degree of positivity (19)

HIGH Positive	Samples > 80 GPL/MPL units
MEDIUM Positive	Samples between ~20 and 80 GPL/MPL units
LOW Positive	Samples between cut-off and ~20 GPL/MPL units
Cut-Off Point	Cut-off values may vary if other experimental conditions or other assay/Kits are used. In those instances if not indicated in the package insert by the manufacturer, each laboratory must determined its own "cut-off" point.

10 – LIMITATIONS

- Diagnosis of the Antiphospholipid Syndrome (APS) cannot be based solely on a positive aCL antibody test.
- Criteria for this diagnosis include a history of one of the following clinical features: thrombosis, pregnancy loss or thrombocytopenia, combined with a positive aCL ELISA test and/or an anti-$\beta_2$glycoprotein I test and/or positive lupus anticoagulant test (8-10).
- Patients may have positive lupus anticoagulant but negative aCL tests; hence, both tests should be performed in patients suspected of having the Antiphospholipid Syndrome (APS).

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Louisville APL Diagnostics, Inc.

Revision 4 – May 1st, 2020

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