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Title

Semiquantitative interpretation of anticardiolipin and antiβ2glycoprotein I antibodies measured with various analytical platforms: Communication from the ISTH SSC Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibodies

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Abstract

Background Antiβ2glycoprotein I (aβ2GPI) and anticardiolipin (aCL) IgG/IgM show differences in positive/negative agreement and titers between solid phase platforms. Method specific semiquantitative categorization of titers could improve and harmonize the interpretation across platforms.

Aim To evaluate the traditionally 40/80 units thresholds used for aCL and aβ2GPI for categorization into moderate/high positivity with different analytical systems, and to compare with alternative thresholds.

Material and methods aCL and aβ2GPI thresholds were calculated for two automated systems (chemiluminescent immunoassay (CLIA) and multiplex flow immunoassay (MFI)) by ROC-curve analysis on 1108 patient samples, including patients with and without APS, and confirmed on a second population (n=279). Alternatively, regression analysis on diluted standard material was applied to identify thresholds. Thresholds were compared to 40/80 threshold measured by an enzyme linked immunosorbent assay (ELISA). Additionally, likelihood ratios (LR) were calculated.

Results Threshold levels of 40/80 units show poor agreement between ELISA and automated platforms for classification into low/moderate/high positivity, especially for aCL/aβ2GPI IgG. Agreement for semiquantitative interpretation of aPL IgG between ELISA and CLIA/MFI improves with alternative thresholds. LR for aPL IgG increase for thrombotic and obstetric APS based on 40/80 thresholds for ELISA and adapted thresholds for the other systems, but not for IgM.

Conclusion Use of 40/80 units as medium/high thresholds is acceptable for aCL/aβ2GPI IgG ELISA, but not for CLIA and MFI. Alternative semiquantitative thresholds for non-ELISA platforms can be determined by a clinical approach or by using monoclonal antibodies. Semiquantitative reporting of aPL IgM has less impact on increasing probability for APS.

Essentials

-Variability in titer between platforms hampers qualitative classification of aCL/aβ2GPI

-Semiquantitative reporting may harmonize interlaboratory interpretation

-Previously defined 40/80 GPL/MPL for low/medium positivity applies only for ELISA

-Platform-specific thresholds can be calculated by clinical approach or using standard materials

Key words

Antiphospholipid antibodies; classification; immunoassay; risk; thresholds

Introduction

The antiphospholipid syndrome (APS) is an autoimmune disease characterized by occurrence of thrombosis and/or pregnancy morbidity with the persistent presence of antiphospholipid antibodies (aPL) [1]. The current laboratory criteria for APS diagnosis require detection of lupus anticoagulant (LAC) and/or anticardiolipin antibodies (aCL) IgG/IgM and/or antiβ2 glycoprotein I antibodies (aβ2GPI) IgG/IgM on at least two occasions with a minimum period of 12 weeks in-between [1, 2]. Detection of LAC is based on functional phospholipid dependent coagulation assays, while aCL and aβ2GPI detection is based on solid phase immunoassays [3, 4].

The 2006 Sydney classification criteria consider detection of aCL IgG or IgM to be significant if they are present in moderate to high titer in serum or plasma, measured by a standardized enzyme linked immunosorbent assay (ELISA). Moderate to high titer for aCL is defined as >40 GPL or MPL or >99th percentile based on a reference population. Detection of a significant level of aβ2GPI IgG/IgM is defined by a titer >99th percentile based on a reference population [1, 5]. However, the Scientific and Standardization Committee (SSC) from the International Society on Thrombosis and Haemostasis (ISTH) does not advise the use of 40 GPL/MPL as cut-off for solid phase aPL positivity [4]. Assays for detecting aCL and aβ2GPI are subject to significant intra-assay, inter-assay and interlaboratory variation [6-11]. Besides differences in agreement (positivity versus negativity), also large variation in titers have been described and there can be a marked difference between 40 GPL/MPL for aCL and the locally derived 99th percentile [7, 12, 13]. Therefore, it seems impossible to advise one general numeric threshold for classifying solid phase aPL titers as “moderate to high”. Consequently, it is recommended to calculate a laboratory-specific cut-off value for positivity based on a non-parametric 99th percentile of at least 120 reference individuals or to transfer manufacturers’ cut-offs after verification on 20 or more reference individuals [4]. Currently, it is recommended to classify each aCL and aβ2GPI result above the cut-off as positive and to report a numeric value along with the in-house cut-off value [4]. External quality control programs show that qualitative classification into ranges of low/moderate/high differs between platforms, and users ascribe a different classification to an identical numerical test result [9].

On the other hand, semiquantitative reporting of results as “low”, “moderate” or “high” can be useful for the clinician and could improve and harmonize the interpretation of aCL and aβ2GPI titers across multiple assays and laboratories [5, 14, 15]. However, semiquantitative classification of solid phase aPL ranges based on fixed antibody titers is currently not recommended due to high variation and lack of standardization of the laboratory assays [2, 4].

We aimed to evaluate whether traditionally established aCL and aβ2GPI thresholds of 40 and 80 MPL/GPL are appropriate to categorize results as moderate and high titers [1, 5, 14]. We investigated the agreement of low, moderate and high aCL and aβ2GPI IgG/M aPL titers defined by the 40/80 threshold and by different approached thresholds, between the solid phase platforms. We included an ELISA system and two automated analytical systems based on chemiluminescent immunoassay (CLIA) and multiplex flow immunoassay (MFI) principles. Two separate patient populations were included, the first one was a large cohort of well-described APS and non-APS patients, analyzed in parallel for aPL with the three platforms [7]. Results were confirmed on a second daily routine patient population. Additionally, we used standard materials to establish semiquantitative thresholds.

Materials and methods

Patient cohorts

Two patient cohorts were selected. The first cohort consisted of 1108 patient samples collected from eight European medical centers. Sydney clinical classification and ISTH laboratory criteria were followed by the local centers for classification of obstetric APS (n=122) and thrombotic APS (n=259) [1, 2, 4]. A non-APS control population was selected consisting of patients with obstetric complications, not fulfilling the laboratory criteria for APS (non-APS obstetric, n=33); patients with a history of at least one thrombotic event, not fulfilling the laboratory criteria for APS (non-APS thrombosis, n=204); patients with a non-APS autoimmune disease without a history of thrombosis or pregnancy complications (autoimmune disease controls [AID], n=196); patients that were tested for aPL for other reasons than those included in the clinical criteria for APS, for instance subfertility and investigation of prolonged activated partial thromboplastin time (aPTT) (controls, n=194); and patients with history of a normal pregnancy and no history of thrombosis (normal pregnancy, n=100).

The second patient cohort was analyzed to validate the semiquantitative ranges defined in cohort 1, by assessment of kappa agreement. This cohort consisted of 279 patient samples, routinely analyzed with CLIA for aCL or aβ2GPI at Ghent University Hospital and with at least one solid phase aPL >20 U/mL (aCL IgG/IgM and/or aβ2GPI IgG/IgM).

All patient samples were stored at -80°C until analysis. The study was approved by the local ethical committees.

Laboratory assays

Samples from the first cohort were analyzed for aCL IgG, aCL IgM, aβ2GPI IgG, and aβ2GPI IgM at Ghent University Hospital (Ghent, Belgium) with three commercially available solid phase immunoassays: QUANTA Lite ELISA (Inova Diagnostics, San Diego, California, USA) performed manually with BEP IIII System plate reader (Siemens Healthcare, Erlangen, Germany), ACL AcuStar CLIA (Werfen/Instrumentation Laboratories, Bedford, Massachusetts, USA) and BioPlex 2200 MFI (Bio-Rad, Bio-Rad Laboratories, Hercules, California, USA). Two samples of the normal pregnancy category were not analyzed for BioPlex 2200 aCL IgG and aβ2GPI IgG because of insufficient sample volume. The second cohort of samples were analyzed with QUANTA Lite ELISA and ACL AcuStar CLIA only, as BioPlex 2200 MFI was not available anymore in the laboratory during this current study. Analysis was performed according to the manufacturer's instructions. Cut-off values for positivity provided by the manufacturer were transferred upon confirmation in 20 healthy volunteers, following the ISTH-SSC guidelines for solid phase assays [4]. aPL titers were expressed using MPL and GPL units for ELISA aCL IgM and IgG isotypes, respectively, and SMU/SGU for ELISA aβ2GPI IgM/IgG, U/mL for both isotypes CLIA aCL and aβ2GPI, and GPL-U/MPL-U for aCL IgG/IgM and U/mL for aβ2GPI IgG/IgM with MFI.

For calibration of the assays, ACL AcuStar uses an internal standard correlated with so called 'Sapporo standards' EY2C9 (IgM) and HCAL (IgG). Also for the QUANTA Lite ELISA, an internal standard correlated to the Sapporo standards is used for aCL IgG and IgM, while it is not specified for aβ2GPI IgG and IgM. BioPlex 2200 uses an internal reference standard, but is not further specified by the manufacturer.

Defining semiquantitative ranges

Positive solid phase aPL results for both cohorts were classified into semiquantitative categories (low, moderate, high) based on different antibody titer ranges.

Traditionally, distinction between low, moderate and high aPL titers is made using 20, 40 and 80 U/mL or GPL/MPL-U as a cut-off detected with ELISA [1, 5]. ELISA antibody titers of 20-40 GPL/SGU for aCL and aβ2GPI IgG were considered low positive. Titers of 40-80 GPL/SGU were considered moderate positive and a titer of >80 GPL/SGU was considered as high. Equally, for aCL and aβ2GPI IgM isotypes: 20-40 MPL/SMU, 40-80 MPL/SMU, >80 MPL/SMU were considered low, moderate and high positive, respectively. We categorized CLIA and MFI aPL titers with the same numerical thresholds as for the ELISA in cohort 1.

Alternatively, receiver operating characteristics (ROC) analysis was performed with results of all three platforms for the thrombotic test population (n=853) and obstetric test population (n=645) of cohort 1 to determine ROC sensitivity-based thresholds. Thrombotic APS and obstetric APS (defined as above) were

defined as 'disease' in their respective test populations for ROC analysis purposes. Sensitivity and specificity were determined for ELISA aPL levels near the previously defined traditional semiquantitative thresholds of 40 and 80 MPL/GPL/SMU/SGU. Thresholds for aPL results analyzed by CLIA and MFI were determined by considering equal sensitivity for diagnosing thrombotic or obstetric APS. We defined the cut-off value based on sensitivity, knowing that both test systems have comparable sensitivity in our patient cohort [7].

International standard material

Two international standard materials were measured with QUANTA Lite ELISA and ACL AcuStar CLIA for aCL and aβ2GPI, both IgG and IgM. Standard material was not analyzed on BioPlex 2200 MFI as the platform was not available in the laboratory for part of the study. Sapporo standards (Inova Diagnostics, San Diego, California, USA) were measured in a serial dilution series with normal pooled plasma (prepared in-house by mixing citrated plasma from 75 healthy volunteers), as well as a dilution series of Harris standards LAPL-GM-300 (Louisville APL Diagnostics, Texas City, Texas, USA) were measured. Additionally, a human-derived monoclonal antibody (MoAB) EM6 IgG was used in serial dilutions of antibodies (0–250 µg/mL) and analyzed for aCL and aβ2GPI IgG with ELISA, CLIA and MFI [7]. These spiked samples were handled as patient samples and tested in the same conditions. All spiked plasmas were measured in duplicate.

Linear regression analysis was performed with positive results (>20 'units') on both compared platforms. Based on regression equations, a corresponding moderate and high range threshold value was determined for CLIA and/or MFI compared to ELISA (40 and 80 'units').

Statistical analysis

Within the first cohort, two subpopulations for statistical analysis were defined: a thrombotic population (n=853) consisting of thrombotic APS, AID, controls, and non-APS thrombosis; an obstetric population (n=645) consisting of obstetric APS, AID, controls, non-APS obstetric, and normal pregnancy. These two subpopulations include overlap for the AID and control population.

Sensitivity and specificity were determined by ROC analysis at different cut-off levels, defining thrombotic APS and obstetric APS as disease state in the two subpopulations respectively. Positive likelihood ratios (LR) and interval-specific LR were calculated with corresponding 95% confidence interval (CI). Including the samples positive with both assays (ELISA and CLIA/MFI), 2 x 2 contingency tables were constructed at

different thresholds for each subpopulation as well as for the second cohort. Cohen's kappa was calculated to assess inter-platform reliability. aPL results were visually represented in scatter plots. Linear regression was used to evaluate standard materials measured with ELISA and CLIA. Statistical analysis was performed with SPSS Statistics 27 (IBM, Armonk, New York, USA) and MedCalc v15.6.1 (MedCalc Software, Ostend, Belgium).

Results

Results and comparison of semiquantitative thresholds of aPL by solid phase assays in patient cohort 1

All samples of cohort 1 (n= 1108) were analyzed for aCL IgG/IgM and aβ2GPI IgG/IgM with ELISA, CLIA, and MFI. Positivity for aCL IgG, aCL IgM, aβ2GPI IgG, and aβ2GPI IgM with both ELISA and CLIA and both ELISA and MFI are shown in Table 1. A graphical representation comparing ELISA and CLIA titers is presented in Figure 1 (1A-B and 2A-B) for aCL IgG and aβ2GPI IgG and Figure 2 (1A-B and 2A-B) for aCL IgM and aβ2GPI IgM.

Cohen's kappa was calculated based on 2x2 contingency tables (Supporting information Table 2-5), comparing inter-system reliability between ELISA and CLIA or MFI for categorizing results as low-moderate-high by using the different defined thresholds [16]. With focus on titer ranges, titers of samples positive in both platforms were compared.

Traditional semiquantitative ranges

Within the thrombotic test population, IgG aPL kappa values range from -0.06 to 0.23 comparing low-moderate-high with the same numeric threshold levels (20/40/80) for ELISA and CLIA (Table 3A). The kappa values illustrate that there is no to minimal agreement between ELISA and CLIA for determining whether a positive aCL/aβ2GPI IgG sample is considered low, moderate or high positive. Results are comparable for the obstetric population, with kappa values from -0.01 to 0.26 (Table 3B). Considering traditional semiquantitative categories, kappa ranges from 0.29 to 0.43 for aCL IgM and from 0.35 to 0.79 for aβ2GPI IgM within the thrombotic test population for ELISA-CLIA comparison. Kappa ranges from 0.29 to 0.63 for aCL IgM and from 0.64 to 0.90 for aβ2GPI IgM within the obstetric test population.

The reliability assessment between ELISA and MFI (see Supporting information Table 6) shows no agreement (Kappa <0.15) for IgG when traditional 20/40/80 thresholds are applied. Reliability between

ELISA and MFI is comparable to reliability between ELISA and CLIA for aCL IgM using traditional thresholds with kappa ranging from 0.13 to 0.57 when assessing both test populations.

ROC sensitivity-based semiquantitative ranges

ROC analysis was performed and graphic representation of the results are presented in Supporting information Figure 1. All determined aPL moderate-high threshold levels for both the thrombotic and obstetric test population with corresponding sensitivity and specificity are summarized in Table 2 for ELISA and CLIA. For the MFI platform, these results are summarized in Supporting information Table 1. Sensitivity and specificity for thrombotic and obstetric APS were determined at different antibody titers. For example, in the thrombotic test population sensitivity and specificity for diagnosis of thrombotic APS with ELISA aCL IgG is 0.290 and 0.976, respectively (or 29,0% and 97,6%) near the moderate threshold level of 40 GPL. For CLIA aCL IgG equal sensitivity of 0.290 is found at 202 U/mL, and for MFI at 748 GPL-U (Table 2). These titers were then considered as the alternative moderate threshold level for the respective platform aCL IgG when assessing thrombotic APS. Assessing the high threshold, CLIA aCL IgG titer of 492 U/mL and MFI aCL IgG of 1955 GPL-U were obtained.

Cohen's kappa values are higher for IgG aPL when ROC sensitivity-based thresholds are applied for CLIA compared to traditional thresholds with values ranging from 0.36 to 0.69 (minimal to moderate agreement) within the thrombotic test population and kappa ranging from 0.39 to 0.81 (minimal to strong agreement) within the obstetric test population (Table 3). Kappa values for the moderate and high range only slightly improve within the thrombotic test population for aCL IgM, using the ROC sensitivity-based thresholds for CLIA. Remarkably, within the obstetric population kappa values are all lower for both aCL and aβ2GPI IgM if the alternative CLIA thresholds are used (Table 3).

Kappa increases to 0.25-0.67 within the thrombotic test population and to 0.41-0.70 within the obstetric test population when ROC sensitivity-based threshold values for MFI IgG aPL are applied, except for the moderate range for aβ2GPI IgG, where the agreement remains absent (Kappa=0.16). For MFI aPL IgM, kappa only increases in the moderate range (from 0.14 to 0.30) and the high range (from 0.39 to 0.61). For aβ2GPI IgM, intersystem reliability does not improve if alternative thresholds are applied. Results are summarized in Supporting information Table 6.

Results and comparison of semiquantitative thresholds of aPL by solid phase assays in patient cohort 2

Samples of cohort 2 (n=279) were analyzed with ELISA for the aPL that were positive with CLIA.

Twenty-four, 84, 22, and 51 patient samples for aCL IgG, aCL IgM, aβ2GPI IgG, and aβ2GPI IgM, respectively, were included.

The second patient cohort was used to validate the ROC sensitivity-based semiquantitative categories, calculated in cohort 1. Kappa values were calculated for the second patient cohort, based on 2x2 contingency tables (see Supporting Information Table 7). We did not differentiate between thrombotic or obstetric test populations since ROC sensitivity-based cut-off values for CLIA within the thrombotic and obstetric test populations in cohort 1 were comparable. For cohort 2, we used the mean threshold of the thrombotic and obstetric population of cohort 1 for aCL IgG, aβ2GPI IgG and aβ2GPI IgM at moderate and high threshold (Table 4). For aCL IgM remarkably lower kappa values were observed within the obstetric test population for the moderate range (0.48 versus 0.08, for the thrombotic population and obstetric population, respectively), therefore 45-170 U/mL was considered as alternative threshold to apply in the second patient cohort. The low range was fixed at 20-40 as for cohort 1. Data are visualized in Figure 1 (1C and 2C) and Figure 2 (1C and 2C) for aPL IgG and aPL IgM, respectively.

Kappa values range from -0.19 to 0.27 for aCL IgG and aβ2GPI IgG considering traditional 40-80 U/mL (moderate) thresholds. Kappa improves for the low and high range to 0.37-0.64 while kappa remains low (-0.14 and 0.03) for the moderate range when using the alternative moderate and high thresholds for CLIA. Kappa values for the three ranges do not improve when using ROC sensitivity-based thresholds for CLIA aCL and aβ2GPI IgM (Table 4).

Results of aPL by solid phase assays in dilution series of standard material

A graphical representation comparing ELISA and CLIA titers for the dilution series of standard material is presented in Figure 1 (1D and 2D) for aCL IgG and aβ2GPI IgG and Figure 2 (1D and 2D) for aCL IgM and aβ2GPI IgM with corresponding regression curves and equations.

Threshold levels based on regression analysis for standard materials are included in Tables 3-5 and Supporting information Table 8 (ELISA/MFI). For both aCL and aβ2GPI IgG, IgM, Sapporo standards and MoAB EM6 demonstrate higher values for CLIA as compared to Harris standards for the corresponding ELISA value. For instance, an aCL IgG ELISA value of 40 GPL corresponds to 127 U/mL, 259 U/mL and 153 U/mL with CLIA for Harris, Sapporo standards, and MoAb EM6, respectively (see Figure 1). For Harris standards, aβ2GPI IgM, 40 SMU with ELISA corresponds to only 10 U/mL with CLIA.

Accordingly, thresholds obtained for Sapporo standard and MoAB EM6 were closer to the thresholds obtained by ROC curve analysis compared to Harris standards. Cohen's kappa statistics were applied for assessing reliability between ELISA and CLIA for categorizing results of cohort 1 and 2 into low-moderate-

high titers with traditional 20-40-80 thresholds and Sapporo and Harris standard-based thresholds, as well as MoAB EM6-based thresholds (Table 3).

For Sapporo standard-based thresholds, in the thrombotic test population, lower kappa values are observed in the moderate range compared to ROC sensitivity-based threshold levels for aCL IgG/IgM and aβ2GPI IgM, while they are comparable to higher in the obstetric population (see Table 3B). In the second cohort, no agreement was observed for any aPL in the moderate range, comparable to ROC sensitivity-based cut-offs (see Table 4). For aCL IgG, kappa is slightly lower if Sapporo-based thresholds are applied compared to ROC sensitivity-based threshold (kappa 0.53 compared to 0.60) in the thrombotic test population of cohort 1 (Table 3). On the other hand, considering cohort 2, kappa is 0.67 for the low range based on Sapporo standards compared to 0.37 based on ROC sensitivity-based moderate threshold level. For aCL IgM, lower kappa values are observed in the low range using Sapporo based thresholds, while comparable to higher kappa values are observed for the high titer range compared to ROC sensitivity-based thresholds (See Table 3 and 4).

Kappa values for all ranges comparing ELISA/CLIA applying the MoAB EM6 calculated thresholds compare to kappa values applying ROC based thresholds, in the thrombotic as well as the obstetric patient population (Table 3A and 3B) and in cohort 2 (Table 4). Equally, for ELISA/MFI Kappa values are comparable in the thrombotic and obstetric patient population, except for the moderate range for aCL IgG (Supporting information Table 6). For the moderate range in the obstetric population the kappa agreement is higher based on MoAB EM6 ranges compared to ROC-curve based ranges.

Likelihood ratios

Positive LR were assessed for the threshold above the upper limit of the high threshold and for the ranges (interval specific LR) within the thrombotic and obstetric test population of cohort 1 (Table 5). 'Thrombotic APS' and 'obstetric APS' were considered as disease in their respective populations. LR was not assessed in cohort 2 as negative results for CLIA aPL were not included in the study. LR are consistently <1 when the aPL value is <20 GPL/MPL or U/mL. For ELISA aCL IgG, LR increases from 4.6/4.7 in the low range to 6.2 and in the moderate range, and to 27 and 15 if >80 GPL for the thrombotic and obstetric test population, respectively. Considering traditional thresholds (20-40-80 U/mL), LR in the thrombotic test population for CLIA aCL IgG improves from 4.0 (20-40 U/mL) and 5.0 (40-80 U/mL) to 11 (>80 U/mL). LR increases to 15 in the range 202-492 U/mL and up to 16 if aCL IgG >80 U/mL. In the obstetric test population, LR is not significantly higher than 1 in the CLIA aCL IgG range of 20-40 U/mL and 40-80 U/mL, but does increase to 4.3 and 11 if the ranges 20-153 U/mL and 153-455 U/mL are considered.

High LR are observed for ELISA aβ2GPI IgG in the low range for both test populations (8.5 and 6.9) increasing to 15 and 8.6 at the high level threshold of >80 SGU. In contrast, low LR values are found at 20-40 U/mL and 40-80 U/mL for CLIA aβ2GPI IgG, increasing to 3.9 and 3.4 for the low range and 8.4 and 5.0 for the moderate range considering the alternative thresholds. In the high range, LR improves from 7.8 and 6.0 at >80 U/mL to 62 and 11 when thresholds of > 4904 U/mL and > 3355 U/mL are defined for the thrombotic and obstetric test population, respectively.

LR for ELISA aCL IgM mildly increases with higher thresholds within the thrombotic test population from 4.1 at 20-40 MPL to 5.2 at >80 MPL, while for CLIA an increase in LR from 2.9 at 20-40 U/mL to 7.6 at >80 U/mL and 9.2 at >170 U/mL is observed. LR do not increase with higher thresholds within the obstetric test population, neither for ELISA or CLIA. However, the alternative CLIA high threshold of 244 U/mL results in a LR of 26 (95% CI: 3.1-212). For aβ2GPI IgM, LR do not clearly increase with higher thresholds in both test populations for both platforms (Table 5).

LR were also determined for CLIA considering alternative thresholds based on Sapporo and Harris standard dilution series (See Table 5). For aCL IgG ranges based on Sapporo and Harris standard defined thresholds and ROC sensitivity-based thresholds result into comparable LR within the thrombotic test population with LR ranging from 4.0-5.1, 14-15, and 14-16 in the low, moderate, and high range, respectively. Within the obstetric test population, aCL IgG LR ranges from 3.8-4.9, 11-13, 7.3-8.2 in the low, moderate, and high range, respectively.
Equally, for aβ2GPI IgG comparable LR for ROC sensitivity, Sapporo and Harris based thresholds are observed at the moderate threshold (thrombotic and obstetric test population) and high threshold (obstetric test population), but LR differ for levels above the upper limit of the high threshold ranging from 17 to 62 with overlapping CI within the thrombotic test population. aCL IgM shows the highest LR (18, 95%CI: 4.2-79) in the moderate range based on Sapporo standard dilution series (98-120 U/mL) within the thrombotic test population. Within the obstetric test population, aCL IgM reaches significant LR in the moderate range calculated by the Harris standard dilution series only. In the high range, LR are lower based on Harris standard dilution series (4.3) compared to the Sapporo standard dilution series (8.6) and ROC sensitivity-based threshold (26). For aβ2GPI IgM LR are comparable for all defined ranges.

LR for CLIA and MFI were calculated and shown in Table 5 (CLIA) and Supporting information Table 8 (MFI) for MoAB EM6 based ranges. For CLIA, LR are comparable to LR obtained based on ROC-curve based ranges for both IgG aPL in the thrombotic and obstetric population. For MFI, LR based on ROC-curve based ranges are lower in the moderate range, and significantly higher in the high range, compared to the MoAb EM6 based ranges.

Discussion

Semiquantitative reporting of solid phase aPL results could be helpful to standardize interpretation across laboratories and for risk stratification purposes [14, 15]. In clinical guidelines, classification of positive aCL and aβ2GPI titers in "low range" or "moderate-to-high range" is recommended for APS diagnosis and classification, as well as risk profiling. These reports often use thresholds for determining the aCL and/or aβ2GPI low range (20-40 'units') and moderate-to-high range (>40 'units' or >99th percentile) without differentiating between analytical platforms such as ELISA, CLIA and MFI or consider only ELISA [5, 17-19]. Initially, Harris *et al* defined a titer of 20 or 40 GPL/MPL as threshold for moderate aCL IgG/IgM positivity and 80 GPL/MPL as threshold for the high positive range, based on the S-shape of an ELISA calibration curve [14, 20]. Others demonstrated that aCL IgG titers >40 GPL correlated more with APS related clinical events and characteristics compared to positive aCL IgG titers <40 GPL, measured with ELISA [21-23]. Automated platforms using different techniques, such as CLIA and MFI, have some advantages over ELISA, are commercially available as alternative for ELISA, and perform well [24]. However, they also show inter-assay variability and limited numerical agreement with ELISA [7, 11, 12, 25, 26].

Current classification criteria for APS, as well as classification criteria for SLE include aPL [1, 27]. The main purpose of the consensus APS classification criteria is to provide uniform guidelines and patient selection criteria for scientific research, and are not meant for diagnosis, although these same laboratory criteria are fulfilled for the majority of the patients at the time of diagnosis. The description of a threshold between low and moderate/high was added in the Sydney criteria to increase specificity to the diagnosis of APS [1]. In recent years, more and more alternative solid phase assays have been introduced in the laboratories, and the previously defined threshold of 40 GPL or MPL units/mL for aCL antibodies has been proven in our study to stand for ELISA only.
An international multi-disciplinary initiative has been started to develop new classification criteria to identify patients with high likelihood of APS for research purposes, and recognize the difference in semiquantitative reporting between solid phase platforms [28].

In this study we demonstrate that there is poor agreement between ELISA and automated platforms CLIA or MFI for classifying positive samples as being low, moderate or high positive if traditional threshold levels of 40 and 80 'units' are used to discriminate between low-moderate aPL levels and moderate-high levels, respectively. Cohen's kappa values were determined by comparing semiquantitative interpretation of ELISA and CLIA or MFI results, only including positive patient samples on both ELISA and the automated platform (CLIA or MFI) to exclude bias of negative results.

No agreement (Cohen's kappa <0.21) was observed for aβ2GPI IgG comparing both ELISA - CLIA and ELISA – MFI, which was confirmed for CLIA on a second, independent patient cohort. In the thrombotic test population of cohort 1, 87/88 and 86/88 samples positive with ELISA aβGPI IgG had values >80 U/mL with CLIA and MFI, respectively. For aCL IgG, no agreement in semiquantitative classification was observed between ELISA and MFI, while none to minimal agreement was observed for comparing ELISA and CLIA. For aCL IgM and aβ2GPI IgM agreement varied from minimal to weak in the low and moderate range, and was moderate to strong in the high range.

These results confirm high inter-assay titer variability for aCL and aβ2GPI, especially for IgG, and inability of standardized, semiquantitative interpretation of solid phase aPL IgG results based on traditional moderate-high thresholds of 40-80 'units'.

In our population, increasing LR for thrombotic and obstetric APS based on ELISA aCL IgG was observed with increasing thresholds. This means that a sample with aCL IgG 40-80 GPL has a higher probability of thrombotic or obstetric APS diagnosis, compared to samples with aCL IgG 20-40 GPL. Even higher LR were observed when using a threshold of 80 GPL. The same trend was observed in the thrombotic test population for aβ2GPI IgG, but not in the obstetric test population. Increasing LR with increasing autoantibody titers have already been reported for other autoimmune diseases and APS [29, 30]. However, it should be noted that conditions such as other auto-immune diseases can have high solid phase aPL titers as demonstrated in Figure 1 and 2, without having clinical manifestations of APS. Based on our LR analysis, 40 GPL seems an appropriate threshold for discriminating between low and moderate-high aCL and aβ2GPI IgG titers, especially in the setting of thrombotic APS when using QUANTA Lite ELISA.

On the contrary, for aCL IgM and aβ2GPI IgM no notable difference in LR was observed for diagnosis of thrombotic or obstetric APS when QUANTA Lite ELISA thresholds of 40 and 80 MPL/SMU were used. This observation suggests that it is not useful to interpret solid phase aPL IgM antibodies semiquantitatively as there is no different probability in being diagnosed with APS between the defined ranges. The role of IgM

aCL and aβGPI antibodies in APS is debated [31-34], although we have previously demonstrated that there might be added value of testing for aCL and aβ2GPI IgM in women suspected of obstetric APS and thrombotic risk stratification [35].

Different strategies were applied to harmonize semiquantitative interpretation of solid phase aPL. First, a clinical approach applying ROC analysis was used for comparing ELISA with CLIA and MFI as previously suggested by Lakos *et al* for CLIA [23]. High aCL IgG and aβ2GPI IgG titers were observed with CLIA and MFI considering equal sensitivity for thrombotic or obstetric APS diagnosis at 40 and 80 GPL/SGU thresholds for ELISA. This results in alternative ROC sensitivity-based thresholds for CLIA and MFI that were 5 up to 64 times higher compared to 40 and 80 GPL/SGU with ELISA (see Table 2, Supporting information Table 1). On the other hand for aCL IgM and aβ2GPI IgM, rather comparable values were observed with CLIA and MFI. Sensitivity analysis for IgG isotype has shown before to indicate a higher thrombotic risk [36].

LR for aCL and aβ2GPI IgG were significantly higher with ROC sensitivity-based ranges, particularly in the moderate and high range for thrombotic APS, and in the moderate range for obstetric APS. For aCL and aβ2GPI IgM differences in LR were small in thrombotic and obstetric APS, except for the high range in the obstetric population. Ranges defined on a clinical approach, compared by the fixed threshold of 40/80 resulted in significant higher LR, particularly in the moderate and high range for IgG aPL. The threshold obtained by ROC curve analysis for CLIA corresponding to the threshold of 40 for ELISA, was 202 in our cohort, and was higher compared to the one described in literature, being 95 U/mL [23]. Although we observed an increase between ELISA/CLIA in kappa agreement applying the ROC sensitivity-based thresholds in a second independent patient population, this suggests that each laboratory should calculate in-house thresholds. ROC sensitivity-based thresholds seems an appropriate way to calculated low/moderate/high ranges, but the requirement of a large patient population tested by two solid phase assays is not feasible for most labs.

Therefore, we searched for another approach by using both monoclonal standard material (Sapporo standards, MoAB EM6) and polyclonal reference standard material (Harris standards) to compare positive titers by ELISA with CLIA and MFI. We observed differences in aCL/aβ2GPI titer between solid phase methods for the standard materials. Calculated thresholds for Sapporo standards and MoAB EM6 corresponded best with the clinical approach of ROC sensitivity-based thresholds. LR based on MoAB EM6-based thresholds were comparable or higher compared to the LR based on ROC-curve analysis for

IgG aPL, in the thrombotic and obstetric population. Qualitative agreement based on Cohen's kappa statistics between ELISA and CLIA improves for IgG aPL using Sapporo- and EM6-based thresholds based in cohort 1, while the improvement is clearly less for Harris standard-based thresholds. This was verified in cohort 2, for all standard-based thresholds, but a uniformly poor agreement at the moderate range was observed, except for aβ2GPI IgG with Harris-based thresholds. This might suggest that evaluation of thresholds could be population-dependent, however also fewer samples were assessed in cohort 2 compared to cohort 1. Improvement of agreement is also observed for EM6-based thresholds with MFI, however Harris and Sapporo standards were not tested and no second cohort for verification purposes was included. Monoclonal antibodies have the advantage to have reproducibility between batches, although they do not necessarily mirror the polyclonality of antibodies encountered in patient populations. The polyclonal Harris standards are patient-derived reference materials developed for calibration of the aCL assay and not for the aβ2GPI assay. When new Harris standards would be produced and matched with the original Harris calibrators, matching with aβ2GPI will not be performed and is therefore not reproducible across different batches.

Further investigations in existing cohorts should be performed to confirm whether threshold ranges defined on one cohort is interchangeable between laboratories. Each laboratory could determine laboratory- or platform-specific moderate and high thresholds by measuring a dilution series of monoclonal antibodies with ELISA and their platform and calculating corresponding thresholds through linear regression analysis. The manufacturer could play here an important role by providing the thresholds for moderate/high levels of aPL for their system, on the condition that a large representative patient population has been tested with ELISA and their method, and the proper statistical methodology has been used. In analogy with transfer of reference values [4], laboratories can check the ranges provided by the manufacturer.

A limitation of this study is that there is no gold standard or lab-independent method available for defining thrombotic and obstetric APS. Therefore, the case categorization is assay-dependent, which might introduce unavoidable bias especially in ROC and LR analysis. We attempted to reduce this bias by including a large number of samples from eight different centers using varying analytical methods for aPL detection.

In conclusion, we have demonstrated that the use of 40 and 80 units as moderate and high thresholds is acceptable for aCL IgG and aβ2GPI IgG ELISA but cannot be applied to analytical solid phase platforms

with CLIA or MFI methodology. Based on our results, semiquantitative interpretation defining medium/high thresholds of aCL IgM and aβ2GPI IgM does not increase probability of APS diagnosis. Better harmonization of semiquantitative interpretation of aCL IgG and aβ2GPI IgG between ELISA and other analytical platforms can be achieved by using a clinical approach, however this is cumbersome and not feasible for many laboratories. A more accessible method to define platform-specific thresholds, is the calculation of thresholds following comparison of parallel measurement of monoclonal antibodies by an automated solid phase platform and ELISA.

Author contributions

K.M.J. Devreese, W. Chayoua and A. Vandevelde designed the study. K.M.J. Devreese organized the sample collection of the different centers. A. Vandevelde, K.M.J. Devreese, G.W. Moore, J-C. Gris, J. Musiał, S. Zuily and D. Wahl collected samples and identified sample characteristics. Samples were analyzed under supervision of K.M.J. Devreese. A. Vandevelde, W. Chayoua and K.M.J. Devreese interpreted data and performed statistical analyses. A. Vandevelde and K.M.J. Devreese wrote the manuscript. W. Chayoua, B. d. L., G.W. Moore , J-C. Gris, J. Musiał, S. Zuily and D. Wahl critically reviewed the manuscript.

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Conflicts of interest

B. de Laat is an employee of Synapse Research foundation and advisor for Diagnostica STAGO, G.W. Moore was employed at Guy's & St. Thomas' Hospitals at the time of sample collection and reports consultancy fees from Technoclone, D. Wahl reports personal fees, outside the submitted work, from Alexion and GlaxoSmithKline and support to attend scientific meetings from Bayer Healthcare and Leo Pharma. The other authors state that they have no relevant conflict of interest.

References

1. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, PG DEG, Koike T, Meroni PL, Reber G, Shoenfeld Y, Tincani A, Vlachoyiannopoulos PG, Krilis SA. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). Journal of thrombosis and haemostasis. 2006;4:295-306.
2. Devreese KMJ, Ortel TL, Pengo V, de Laat B. Laboratory criteria for antiphospholipid syndrome: communication from the SSC of the ISTH. Journal of thrombosis and haemostasis. 2018;16:809-13.
3. Devreese KMJ, de Groot PG, de Laat B, Erkan D, Favaloro EJ, Mackie I, Martinuzzo M, Ortel TL, Pengo V, Rand JH, Tripodi A, Wahl D, Cohen H. Guidance from the Scientific and Standardization Committee for lupus anticoagulant/antiphospholipid antibodies of the International Society on Thrombosis and Haemostasis: Update of the guidelines for lupus anticoagulant detection and interpretation. Journal of thrombosis and haemostasis. 2020;18:2828-39.
4. Devreese KM, Pierangeli SS, de Laat B, Tripodi A, Atsumi T, Ortel TL. Testing for antiphospholipid antibodies with solid phase assays: guidance from the SSC of the ISTH. Journal of thrombosis and haemostasis. 2014;12:792-5.
5. Garcia D, Erkan D. Diagnosis and Management of the Antiphospholipid Syndrome. The New England journal of medicine. 2018;378:2010-21.
6. Wong R, Favaloro E, Pollock W, Wilson R, Hendle M, Adelstein S, Baumgart K, Homes P, Smith S, Steele R, Sturgess A, Gillis D. A multi-centre evaluation of the intra-assay and inter-assay variation of commercial and in-house anti-cardiolipin antibody assays. Pathology. 2004;36:182-92.
7. Chayoua W, Kelchtermans H, Moore GW, Gris JC, Musial J, Wahl D, Zuily S, Gianniello F, Fontana P, Remijn J, Urbanus RT, de Laat B, Devreese KMJ. Detection of Anti-Cardiolipin and Anti-beta2glycoprotein I Antibodies Differs between Platforms without Influence on Association with Clinical Symptoms. Thrombosis and haemostasis. 2019;119:797-806.
8. Devreese KM. Antiphospholipid antibody testing and standardization. International journal of laboratory hematology. 2014;36:352-63.
9. Favaloro EJ, Wheatland L, Jovanovich S, Roberts-Thomson P, Wong RC. Internal quality control and external quality assurance in testing for antiphospholipid antibodies: Part I--Anticardiolipin and anti-beta2-glycoprotein I antibodies. Seminars in thrombosis and hemostasis. 2012;38:390-403.
10. Tincani A, Allegri F, Sanmarco M, Cinquini M, Taglietti M, Balestrieri G, Koike T, Ichikawa K, Meroni P, Boffa MC. Anticardiolipin antibody assay: a methodological analysis for a better consensus in routine

determinations--a cooperative project of the European Antiphospholipid Forum. Thrombosis and haemostasis. 2001;86:575-83.

11. Devreese KMJ. Testing for antiphospholipid antibodies: Advances and best practices. International journal of laboratory hematology. 2020;42 Suppl 1:49-58.

12. Montaruli B, De Luna E, Erroi L, Marchese C, Mengozzi G, Napoli P, Nicolo C, Romito A, Bertero MT, Sivera P, Migliardi M. Analytical and clinical comparison of different immunoassay systems for the detection of antiphospholipid antibodies. International journal of laboratory hematology. 2016;38:172-82.

13. Devreese KM, Van Hoecke F. Anticardiolipin and anti-beta2glycoprotein-I antibody cut-off values in the diagnosis of antiphospholipid syndrome: more than calculating the in-house 99th percentiles, even for new automated assays. Thrombosis research. 2011;128:598-600.

14. Harris EN, Pierangeli SS. Revisiting the anticardiolipin test and its standardization. Lupus. 2002;11:269-75.

15. Devreese KMJ. How to Interpret Antiphospholipid Laboratory Tests. Current rheumatology reports. 2020;22:38.

16. McHugh ML. Interrater reliability: the kappa statistic. Biochemia medica. 2012;22:276-82.

17. Sayar Z, Moll R, Isenberg D, Cohen H. Thrombotic antiphospholipid syndrome: A practical guide to diagnosis and management. Thrombosis research. 2021;198:213-21.

18. Schreiber K, Sciascia S, de Groot PG, Devreese K, Jacobsen S, Ruiz-Irastorza G, Salmon JE, Shoenfeld Y, Shovman O, Hunt BJ. Antiphospholipid syndrome. Nature reviews Disease primers. 2018;4:17103.

19. Tektonidou MG, Andreoli L, Limper M, Amoura Z, Cervera R, Costedoat-Chalumeau N, Cuadrado MJ, Dorner T, Ferrer-Oliveras R, Hambly K, Khamashta MA, King J, Marchiori F, Meroni PL, Mosca M, Pengo V, Raio L, Ruiz-Irastorza G, Shoenfeld Y, Stojanovich L, et al. EULAR recommendations for the management of antiphospholipid syndrome in adults. Annals of the rheumatic diseases. 2019;78:1296-304.

20. Harris EN. Special report. The Second International Anti-cardiolipin Standardization Workshop/the Kingston Anti-Phospholipid Antibody Study (KAPS) group. American journal of clinical pathology. 1990;94:476-84.

21. Levine SR, Salowich-Palm L, Sawaya KL, Perry M, Spencer HJ, Winkler HJ, Alam Z, Carey JL. IgG anticardiolipin antibody titer > 40 GPL and the risk of subsequent thrombo-occlusive events and death. A prospective cohort study. Stroke. 1997;28:1660-5.

22. Finazzi G. The Italian Registry of Antiphospholipid Antibodies. Haematologica. 1997;82:101-5.

23. Lakos G, Bentow C, Mahler M. A Clinical Approach for Defining the Threshold between Low and Medium Anti-Cardiolipin Antibody Levels for QUANTA Flash Assays. Antibodies. 2016;5.
24. Devreese KM, Poncet A, Lindhoff-Last E, Musial J, de Moerloose P, Fontana P. A multicenter study to assess the reproducibility of antiphospholipid antibody results produced by an automated system. Journal of thrombosis and haemostasis. 2017;15:91-5.
25. Van Hoecke F, Persijn L, Decavele AS, Devreese K. Performance of two new, automated chemiluminescence assay panels for anticardiolipin and anti-beta2-glycoprotein I antibodies in the laboratory diagnosis of the antiphospholipid syndrome. International journal of laboratory hematology. 2012;34:630-40.
26. Grossi V, Infantino M, Benucci M, Li Gobbi F, Bandinelli F, Damiani A, Bodio C, Borghi MO, Mahler M, Aure MA, Bentow C, Manfredi M. Two Novel Technologies for the Detection of Anti-cardiolipin and Anti beta2-Glycoprotein Antibodies in the Real Life: Chemiluminescent in Comparison to the Addressable Laser Bead Immunoassays. Immunological investigations. 2020;49:58-68.
27. Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, Smolen JS, Wofsy D, Boumpas DT, Kamen DL, Jayne D, Cervera R, Costedoat-Chalumeau N, Diamond B, Gladman DD, Hahn B, Hiepe F, Jacobsen S, Khanna D, Lerstrom K, et al. 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. Arthritis & rheumatology. 2019;71:1400-12.
28. Barbhaiya M, Zuily S, Ahmadzadeh Y, Amigo MC, Avcin T, Bertolaccini ML, Branch DW, de Jesus G, Devreese KMJ, Frances C, Garcia D, Guillemin F, Levine SR, Levy RA, Lockshin MD, Ortel TL, Seshan SV, Tektonidou M, Wahl D, Willis R, et al. Development of New International Antiphospholipid Syndrome Classification Criteria Phase I/II Report: Generation and Reduction of Candidate Criteria. Arthritis care & research. 2020.
29. Bossuyt X, Rasmussen N, van Paassen P, Hellmich B, Baslund B, Vermeersch P, Blockmans D, Cohen Tervaert JW, Csernok E, Damoiseaux J. A multicentre study to improve clinical interpretation of proteinase-3 and myeloperoxidase anti-neutrophil cytoplasmic antibodies. Rheumatology. 2017;56:1533-41.
30. Escalante A, Brey RL, Mitchell BD, Jr., Dreiner U. Accuracy of anticardiolipin antibodies in identifying a history of thrombosis among patients with systemic lupus erythematosus. The American journal of medicine. 1995;98:559-65.
31. Kelchtermans H, Pelkmans L, de Laat B, Devreese KM. IgG/IgM antiphospholipid antibodies present in the classification criteria for the antiphospholipid syndrome: a critical review of their association with thrombosis. Journal of thrombosis and haemostasis. 2016;14:1530-48.

32. Galli M, Reber G, de Moerloose P, de Groot PG. Invitation to a debate on the serological criteria that define the antiphospholipid syndrome. Journal of thrombosis and haemostasis. 2008;6:399-401.
33. Pengo V. A contribution to the debate on the laboratory criteria that define the antiphospholipid syndrome. Journal of thrombosis and haemostasis. 2008;6:1048-9.
34. Del Ross T, Ruffatti A, Cuffaro S, Tonello M, Calligaro A, Favaro M, Facchinetti M, Hoxha A, Punzi L. The clinical relevance of the IgM isotype of antiphospholipid antibodies in the vascular antiphospholipid syndrome. Thrombosis research. 2015;136:883-6.
35. Chayoua W, Kelchtermans H, Gris JC, Moore GW, Musial J, Wahl D, de Groot PG, de Laat B, Devreese KMJ. The (non-)sense of detecting anti-cardiolipin and anti-beta2glycoprotein I IgM antibodies in the antiphospholipid syndrome. Journal of thrombosis and haemostasis. 2020;18:169-79.
36. Zuily S, de Laat B, Guillemin F, Kelchtermans H, Magy-Bertrand N, Desmurs-Clavel H, Lambert M, Poindron V, de Maistre E, Dufrost V, Risse J, Shums Z, Norman GL, de Groot PG, Lacolley P, Lecompte T, Regnault V, Wahl D. Anti-Domain I beta2-Glycoprotein I Antibodies and Activated Protein C Resistance Predict Thrombosis in Antiphospholipid Syndrome: TAC(I)T Study. The journal of applied laboratory medicine. 2020;5:1242-52.

Table 1 Distribution of antiphospholipid antibody values across different analytical platforms.

		CLIA		MFI	
	aCL IgG	**-**	**+**	**-**	**+**
ELISA	**-**	894	54	874	72
	+	21	139	19	141
	aCL IgM	**-**	**+**	**-**	**+**
ELISA	**-**	918	52	943	27
	+	38	100	59	79
	aβ2GPI IgG	**-**	**+**	**-**	**+**
ELISA	**-**	856	138	886	106
	+	4	110	4	110
	aβ2GPI IgM	**-**	**+**	**-**	**+**
ELISA	**-**	972	10	964	18
	+	37	89	31	95

Abbreviations: ELISA: enzyme-linked immunosorbent assay, CLIA: chemiluminescent immunoassay, MFI: multiplex flow immunoassay, aCL: anticardiolipin, aβ2GPI: anti-Beta2-glycoprotein I.

Table 2 Threshold levels with sensitivity and specificity based on ROC analysis

	ELISA			CLIA		
	Threshold[a]	**Sensitivity**	**Specificity**	**Threshold[b]**	**Sensitivity**	**Specificity**
A. Thrombotic test population						
aCL IgG						
Moderate	**39**	0,290	0,976	**202**	0,290	0,981
High	**78**	0,185	0,993	**492**	0,185	0,988
aCL IgM						
Moderate	**40**	0,170	0,968	**45**	0,170	0,97
High	**82**	0,062	0,988	**170**	0,062	0,993
aβ2GPI IgG						
Moderate	**39**	0,189	0,985	**1959**	0,189	0,988
High	**80**	0,104	0,993	**4904**	0,104	0,998
aβ2GPI IgM						
Moderate	**40**	0,181	0,968	**31**	0,181	0,973
High	**79**	0,100	0,983	**66**	0,100	0,983
B. Obstetric test population						
aCL IgG						
Moderate	**39**	0,221	0,975	**153**	0,221	0,975
High	**82**	0,115	0,992	**455**	0,115	0,985
aCL IgM						
Moderate	**39**	0,123	0,964	**46**	0,123	0,966
High	**74**	0,049	0,987	**244**	0,049	0,998
aβ2GPI IgG						
Moderate	**41**	0,123	0,983	**1552**	0,123	0,983
High	**81**	0,066	0,994	**3355**	0,066	0,994
aβ2GPI IgM						
Moderate	**40**	0,131	0,966	**33**	0,131	0,971
High	**74**	0,090	0,981	**59**	0,090	0,981

[a] Units: GPL/MPL for aCL IgG/IgM, SGU/SMU for aβ2GPI IgG/IgM; [b] Units: U/mL; *Abbreviations ELISA: enzyme-linked immunosorbent assay, CLIA: Chemiluminescent immunoassay, aCL: anticardiolipin, aβ2GPI: anti-Beta2-glycoprotein I.*

1

2

Table 3 ELISA and CLIA Cohen's Kappa values in cohort 1

3.A THROMBOTIC TEST POPULATION

Threshold		**Range 1** [a] *40/80*	**Kappa 1**	**Range 2** [a] *ROC based*	**Kappa 2**	**Range 3** [a] *Sapporo based*	**Kappa 3**	**Range 4** [a] *Harris based*	**Kappa 4**	**Range 5** [a] *MoAB EM6 based*	**Kappa 5**
Level	**System**	**aCL IgG (n=105)**									
Low	ELISA CLIA	20-40 20-40	**0.23**	20-39 20-202	**0.60**	20-40 20-259	**0.53**	20-40 20-127	**0.51**	20-40 20-153	**0.60**
Moderate	ELISA CLIA	40-80 40-80	**-0.06**	39-78 202-492	**0.36**	40-80 259-592	**0.16**	40-80 127-244	**0.21**	40-80 153-380	**0.38**
High	ELISA CLIA	>80 >80	**0.18**	>78 >492	**0.66**	>80 >592	**0.62**	>80 >244	**0.51**	>80 >380	**0.64**
		aCL IgM (n=76)									
Low	ELISA CLIA	20-40 20-40	**0.40**	20-40 20-45	**0.43**	20-40 20-98	**0.22**	20-40 20-23	**0.08**	/[d]	**/**
Moderate	ELISA CLIA	40-80 40-80	**0.29**	40-82 45-170	**0.48**	40-80 98-210	**0.08**	40-80 23-65	**-0.01**	/[d]	**/**
High	ELISA CLIA	>80 >80	**0.43**	>82 >170	**0.67**	>80 >210	**0.64**	>80 >65	**0.31**	/[d]	**/**
		aβ2GPI IgG (n=88)									
Low	ELISA CLIA	20-40 20-40	**/** [b]	20-39 20-1959	**0.51**	20-40 20-2362	**0.46**	20-40 20-833	**0.15**	20-40 20-1519	**0.47**

Moderate	ELISA CLIA	40-80 40-80	**-0.02**	39-80 1959-4904	**0.41**	40-80 2362-3693	**0.40**	40-80 833-1650	**-0.03**	40-80 1519-3365	**0.43**
High	ELISA CLIA	>80 >80	**0.01**	>80 >4904	**0.69**	>80 >3693	**0.76**	>80 >1650	**0.36**	>80 >3365	**0.69**
		aβ2GPI IgM (n=69)									
Low	ELISA CLIA	20-40 20-40	**0.40**	20-40 20-31	**0.36**	20-40 20-44	**0.41**	20-40 <20	**/**[c]	/[d]	**/**
Moderate	ELISA CLIA	40-80 40-80	**0.35**	40-79 31-66	**0.49**	40-80 44-106	**0.16**	40-80 10-33	**/**[c]	/[d]	**/**
High	ELISA CLIA	>80 >80	**0.79**	>79 >66	**0.62**	>80 >106	**0.61**	>80 >33	**/**[c]	/[d]	**/**

[a] Units ELISA: GPL/MPL for aCL IgG/IgM, SGU/SMU for aβ2GPI IgG/IgM, CLIA: U/mL. [b] Cohen's Kappa cannot be calculated as there are no values in the specified range for CLIA assay.

[c] Cohen's Kappa not calculated as moderate CLIA threshold was <20 U/mL. [d]Not applicable. Kappa 1, 2, 3, 4, and 5 correspond to Cohen's kappa value considering identical classification of samples as being low/moderate/high based on the analytical system-specific 'ranges 1' for 20/40/80 thresholds, 'ranges 2' for ROC sensitivity-based thresholds and 'ranges 3' for Sapporo standard based thresholds, 'ranges 4' for Harris standard based thresholds, and 'ranges 5' for EM6 monoclonal antibody based thresholds, respectively. Interpretation Cohen's kappa (level of agreement): <0.21 (red): none, 0.21-0.39 (dark orange): minimal, 0.40-0.59 (light orange): weak, 0.60-0.79 (light green): moderate, 0.80-0.90 (green): strong, >0.90 (dark green): almost perfect [16]. *Abbreviations: ELISA: enzyme-linked immunosorbent assay, CLIA: chemiluminescent immunoassay, aCL: anticardiolipin, aβ2GPI: anti-Beta2-glycoprotein I.*

Table 3 *(continued)* ELISA and CLIA Cohen's Kappa values in cohort 1

3.B OBSTETRIC TEST POPULATION

Threshold		Range 1 [a] *40/80*	Kappa 1	Range 2 [a] *ROC based*	Kappa 2	Range 3 [a] *Sapporo based*	Kappa 3	Range 4 [a] *Harris based*	Kappa 4	Range 5 [a] *MoAB EM6 based*	Kappa 5
Level	**System**	**aCL IgG (n=52)**									
Low	ELISA CLIA	20-40 20-40	**0.26**	20-39 20-153	**0.68**	20-40 20-259	**0.71**	20-40 20-127	**0.76**	20-40 20-153	**0.68**
Moderate	ELISA CLIA	40-80 40-80	**-0.01**	39-82 153-455	**0.39**	40-80 259-592	**0.51**	40-80 127-244	**0.07**	40-80 153-380	**0.34**
High	ELISA CLIA	>80 >80	**0.13**	>82 >455	**0.71**	>80 >592	**0.75**	>80 >244	**0.49**	>80 >380	**0.68**
		aCL IgM (n=44)									
Low	ELISA CLIA	20-40 20-40	**0.36**	20-39 20-46	**0.43**	20-40 20-98	**0.34**	20-40 20-23	**0.10**	/[d]	**/**
Moderate	ELISA CLIA	40-80 40-80	**0.29**	39-74 46-244	**0.08**	40-80 98-210	**-0.05**	40-80 23-65	**-0.03**	/[d]	**/**
High	ELISA CLIA	>80 >80	**0.63**	>74 >244	**0.28**	>80 >210	**0.48**	>80 >65	**0.44**	/[d]	**/**
		aβ2GPI IgG (n=35)									
Low	ELISA CLIA	20-40 20-40	**/** [b]	20-41 20-1552	**0.81**	20-40 20-2362	**0.55**	20-40 20-833	**0.38**	20-40 20-1519	**0.68**
Moderate	ELISA CLIA	40-80 40-80	**/** [b]	41-81 1552-3355	**0.55**	40-80 2362-3693	**0.41**	40-80 833-1650	**-0.22**	40-80 1519-3365	**0.44**

High	ELISA CLIA	>80 >80	**/** [b]	>81 >3355	**0.74**	>80 >3693	**0.86**	>80 >1650	**0.47**	>80 >3365	**0.74**
		aβ2GPI IgM (n=39)									
Low	ELISA CLIA	20-40 20-40	**0.68**	20-40 20-33	**0.62**	20-40 20-44	**0.68**	20-40 <20	**/**[c]	/[d]	**/**
Moderate	ELISA CLIA	40-80 40-80	**0.64**	40-74 33-59	**0.40**	40-80 44-106	**0.44**	40-80 10-33	**/**[c]	/[d]	**/**
High	ELISA CLIA	>80 >80	**0.90**	>74 >59	**0.74**	>80 >106	**0.73**	>80 >33	**/**[c]	/[d]	**/**

[a] Units ELISA: GPL/MPL for aCL IgG/IgM, SGU/SMU for aβ2GPI IgG/IgM, CLIA: U/mL. [b] Cohen's Kappa cannot be calculated as there are no values in the specified range for CLIA assay.

[c] Cohen's Kappa not calculated as moderate CLIA threshold was <20 U/mL. [d]Not applicable. Kappa 1, 2, 3, 4, and 5 correspond to Cohen's kappa value considering identical classification of samples as being low/moderate/high based on the analytical system-specific 'ranges 1' for 20/40/80 thresholds, 'ranges 2' for ROC sensitivity-based thresholds and 'ranges 3' for Sapporo standard based thresholds, 'ranges 4' for Harris standard based thresholds, and 'ranges 5' for EM6 monoclonal antibody based thresholds, respectively. Interpretation Cohen's kappa (level of agreement): <0.21 (red): none, 0.21-0.39 (dark orange): minimal, 0.40-0.59 (light orange): weak, 0.60-0.79 (light green): moderate, 0.80-0.90 (green): strong, >0.90 (dark green): almost perfect [16]. *Abbreviations: ELISA: enzyme-linked immunosorbent assay, CLIA: chemiluminescent immunoassay, aCL: anticardiolipin, aβ2GPI: anti-Beta2-glycoprotein I.*

Table 4 ELISA and CLIA Cohen's Kappa values in cohort 2

	Range 1 [a]	**Kappa 1**	**Range 2** [a]	**Kappa 2**	**Range 3** [a]	**Kappa 3**	**Range 4** [a]	**Kappa 4**	**Range 5** [a]	**Kappa 5**
Threshold	*40/80*		*ROC based*		*Sapporo based*		*Harris based*		*MoAB EM6 based*	

Level	System									
		aCL IgG (n=24)								
Low	ELISA CLIA	20-40 20-40	**-0.16**	20-40 20-178	**0.37**	20-40 20-259	**0.67**	20-40 20-127	**0.27**	20-40 20-153	**0.37**
Moderate	ELISA CLIA	40-80 40-80	**-0.19**	40-80 178-474	**-0.14**	40-80 259-592	**0.05**	40-80 127-244	**-0.26**	40-80 153-380	**-0.29**
High	ELISA CLIA	>80 >80	**0.27**	>80 >474	**0.64**	>80 >592	**0.55**	>80 >244	**0.51**	>80 >380	**0.66**
		aCL IgM (n=84)								
Low	ELISA CLIA	20-40 20-40	**0.36**	20-40 20-45	**0.45**	20-40 20-98	**0.25**	20-40 20-23	**0.13**	/[d]	**/**
Moderate	ELISA CLIA	40-80 40-80	**0.24**	40-80 45-170	**0.21**	40-80 98-210	**-0.05**	40-80 23-65	**0.10**	/[d]	**/**
High	ELISA CLIA	>80 >80	**0.59**	>80 >170	**0.52**	>80 >210	**0.55**	>80 >65	**0.52**	/[d]	**/**
		aβ2GPI IgG (n=22)								
Low	ELISA CLIA	20-40 20-40	**-0.09**	20-40 20-1756	**0.57**	20-40 20-2362	**0.49**	20-40 20-833	**0.71**	20-40 20-1519	**0.57**
Moderate	ELISA CLIA	40-80 40-80	**0.18**	40-80 1756-4130	**0.03**	40-80 2362-3693	**-0.15**	40-80 833-1650	**0.58**	40-80 1519-3365	**0.10**
High	ELISA CLIA	>80 >80	**0.04**	>80 >4130	**0.60**	>80 >3693	**0.70**	>80 >1650	**0.81**	>80 >3365	**0.70**
		aβ2GPI IgM (n=51)								

Low	ELISA CLIA	20-40 20-40	**0.38**	20-40 20-32	**0.48**	20-40 20-44	**0.33**	20-40 <20	**/**[c]	/[d]	**/**
Moderate	ELISA CLIA	40-80 40-80	**-0.02**	40-80 32-63	**0.12**	40-80 44-106	**0.11**	40-80 10-33	**/**[c]	/[d]	**/**
High	ELISA CLIA	>80 >80	**0.62**	>80 >63	**0.63**	>80 >106	**0.61**	>80 >33	**/**[c]	/[d]	**/**

[a] Units ELISA: GPL/MPL for aCL IgG/IgM, SGU/SMU for aβ2GPI IgG/IgM, CLIA: U/mL. [b] Cohen's Kappa cannot be calculated as there are no values in the specified range for CLIA assay.

[c] Cohen's Kappa not calculated as moderate CLIA threshold was <20 U/mL. [d]Not applicable. Kappa 1, 2, 3, 4, and 5 correspond to Cohen's kappa value considering identical classification of samples as being low/moderate/high based on the analytical system-specific 'ranges 1' for 20/40/80 thresholds, 'ranges 2' for ROC sensitivity-based thresholds and 'ranges 3' for Sapporo standard based thresholds, 'ranges 4' for Harris standard based thresholds, and 'ranges 5' for EM6 monoclonal antibody based thresholds, respectively. Interpretation Cohen's kappa (level of agreement): <0.21 (red): none, 0.21-0.39 (dark orange): minimal, 0.40-0.59 (light orange): weak, 0.60-0.79 (light green): moderate, 0.80-0.90 (green): strong, >0.90 (dark green): almost perfect [16]. *Abbreviations: ELISA: enzyme-linked immunosorbent assay, CLIA: chemiluminescent immunoassay, aCL: anticardiolipin, aβ2GPI: anti-Beta2-glycoprotein I.*

TABLE 5 Likelihood ratios for antiphospholipid antibody titer ranges

aCL IgG	Cohort 1 Thrombotic test population (n=853)				Cohort 1 Obstetric test population (n=645)			
System	**Range**	**LR+**	**95% CI**		**Range**	**LR+**	**95% CI**	
ELISA	0-20	**0.66**	0.60	0.72	0-20	**0.72**	0.64	0.81
CLIA		**0.60**	0.54	0.67		**0.66**	0.58	0.76
ELISA	20-40	**4.6**	2.3	9.3	20-40	**4.7**	2.0	11
CLIA		**4.0**	1.7	9.5		**2.7**	0.89	8.0
ROC	20-202	**4.0**	2.4	6.8	20-153	**4.3**	2.3	7.8
Sapporo	20-259	**5.1**	3.2	8.3	20-259	**4.9**	2.8	8.3
Harris	20-127	**3.3**	1.8	5.9	20-127	**3.8**	2.0	7.3
EM6	20-153	**4.0**	2.3	6.9	20-153	**4.3**	2.3	7.8
ELISA	40-80	**6.2**	3.0	13	40-80	**6.2**	2.7	14
CLIA		**5.0**	1.8	14		**2.6**	0.62	11
ROC	202-492	**15**	5.5	44	153-455	**11**	4.1	31
Sapporo	259-592	**14**	4.1	46	259-592	**13**	3.5	47
Harris	127-244	**15**	4.3	49	127-244	**13**	3.5	47
EM6	153-380	**11**	4.2	29	153-380	**10**	3.7	13
ELISA	>80	**27**	9.8	74	>80	**15**	5.0	45
CLIA		**11**	6.5	17		**8.6**	5.1	14
ROC	>492	**16**	7.2	34	>455	**7.5**	3.2	17
Sapporo	>592	**15**	6.7	32	>592	**7.3**	3.0	18
Harris	>244	**14**	7.5	26	>244	**8.2**	4.1	17
EM6	>380	**16**	7.6	33	>380	**8.0**	3.5	19
aCL IgM	**Cohort 1 Thrombotic test population (n=853)**				**Cohort 1 Obstetric test population (n=645)**			
System	**Range**	**LR+**	**95% CI**		**Range**	**LR+**	**95% CI**	
ELISA	0-20	**0.77**	0.71	0.83	0-20	**0.80**	0.72	0.89
CLIA		**0.76**	0.70	0.83		**0.81**	0.73	0.91
ELISA	20-40	**4.1**	2.2	7.6	20-40	**4.3**	2.2	8.3
CLIA		**2.9**	1.7	5.1		**2.7**	1.4	5.2
ROC	20-45	**2.9**	1.8	4.9	20-46	**2.8**	1.6	5.0
Sapporo	20-98	**3.0**	2.0	4.4	20-98	**2.5**	1.6	4.1
Harris	20-23	**1.4**	0.3	5.7	20-23	**4.3**	1.1	17
ELISA	40-80	**5.4**	2.8	10	40-80	**3.6**	1.6	8.1
CLIA		**3.4**	1.7	6.7		**3.0**	1.3	6.8
ROC	45-170	**4.6**	2.5	8.6	46-244	**2.0**	0.89	4.6

Sapporo	98-120	**18**	4.2	79	98-120	**4.3**	0.61	30
Harris	23-65	**3.3**	2.0	5.3	23-65	**2.3**	1.3	4.2
ELISA	>80	**5.2**	2.2	13	>80	**3.1**	0.99	9.5
CLIA		**7.6**	3.7	16		**3.8**	1.5	9.7
ROC	>170	**9.2**	3.1	27	>244	**26**	3.1	212
Sapporo	>210	**8.4**	2.4	30	>210	**8.6**	2.2	34
Harris	>65	**6.9**	3.6	13	>65	**4.3**	2.0	9

ROC signifies the method for determining ranges with ROC based sensitivity for CLIA, *Sapporo*, *Harris, and EM6* signify the method for ranges determination based on linear regression with the respective standards for CLIA. LR+ denotes the positive likelihood ratio for diagnosis of thrombotic APS and obstetric APS in the thrombotic test population and obstetric test population, respectively. *Abbreviations: aCL: anticardiolipin, LR+: positive likelihood ratio, CI: confidence interval, ELISA: enzyme-linked immunosorbent assay, CLIA: chemiluminescent immunoassay, aβ2GPI: anti-Beta2-glycoprotein I.*

Table 5 (continued) Likelihood ratios for antiphospholipid antibody titer ranges

aβ2GPI IgG	Cohort 1 Thrombotic test population (n=853)				Cohort 1 Obstetric test population (n=645)			
System	**Range**	**LR+**	**95% CI**		**Range**	**LR+**	**95% CI**	
ELISA	0-20	**0.73**	0.68	0.79	0-20	**0.83**	0.77	0.91
CLIA		**0.53**	0.47	0.60		**0.64**	0.55	0.75
ELISA	20-40	**8.5**	3.7	19	20-40	**6.9**	2.3	21
CLIA		**1.7**	0.8	3.6		**0.57**	0.13	2.5
ROC	20-1959	**3.9**	2.8	5.3	20-1552	**3.4**	2.3	5.0
Sapporo	20-2362	**4.0**	2.9	5.4	20-2362	**3.4**	2.4	4.9
Harris	20-833	**2.8**	1.9	3.9	20-833	**2.7**	1.8	4.1
EM6	20-1519	**3.7**	2.7	5.2	20-1519	**3.2**	2.2	4.7
ELISA	40-80	**10**	3.9	26	40-80	**6.0**	1.9	19
CLIA		**3.2**	1.4	7.1		**2.4**	0.81	7.0
ROC	1959-4904	**8.4**	3.5	20	1552-3355	**5.0**	1.7	15
Sapporo	2362-3693	**11**	2.5	52	2362-3693	**11**	2.1	55
Harris	833-1650	**17**	8.0	38	833-1650	**12.9**	3.5	47
EM6	1519-3365	**7**	2.9	18	1519-3665	**6.4**	2.3	18
ELISA	>80	**15**	5.5	44	>80	**8.6**	2.6	28
CLIA		**7.8**	5.4	11		**6.0**	4.0	9.1
ROC	>4904	**62**	8.5	453	>3355	**11**	3.1	42
Sapporo	>3693	**25**	7.8	82	>3693	**8.6**	2.2	34
Harris	>1650	**17**	8.0	38	>1650	**8.6**	3.5	21
EM6	>3365	**28**	8.6	89	>3365	**11**	3.1	42
aβ2GPI IgM	**Cohort 1 Thrombotic test population (n=853)**				**Cohort 1 Obstetric test population (n=645)**			
System	**Range**	**LR+**	**95% CI**		**Range**	**LR+**	**95% CI**	
ELISA	0-20	**0.78**	0.73	0.84	0-20	**0.81**	0.73	0.90
CLIA		**0.81**	0.76	0.87		**0.84**	0.76	0.91
ELISA	20-40	**4.2**	2.0	8.6	20-40	**5.1**	2.3	11
CLIA		**6.4**	2.3	18		**7.1**	2.6	19
ROC	20-31	**6.1**	1.6	23	20-33	**8.6**	2.6	28
Sapporo	20-44	**7.8**	2.9	20.9	20-44	**7.1**	2.6	19.3
Harris	10	**---**	---	---	10	**---**	---	---
ELISA	40-80	**4.8**	2.3	10	40-80	**2.1**	0.7	6.2

CLIA		**11**	4.2	29		**5.1**	1.6	17
ROC	31-66	**8.0**	3.3	20	33-59	**4.3**	1.4	13
Sapporo	44-106	**8.2**	3.6	19	44-106	**4.9**	1.8	13
Harris	10-33	**3.9**	2.3	6.6	10-33	**3.7**	2.1	6.6
ELISA	>80	**6.6**	3.1	14	>80	**5.2**	2.2	12
CLIA		**4.3**	2.0	9.6		**3.8**	1.5	10
ROC	>66	**6.0**	2.9	12	>59	**4.3**	1.8	10
Sapporo	>106	**4.3**	1.7	11	>106	**3.7**	1.3	11
Harris	>33	**6.5**	3.7	11	>33	**4.3**	2.2	8.3

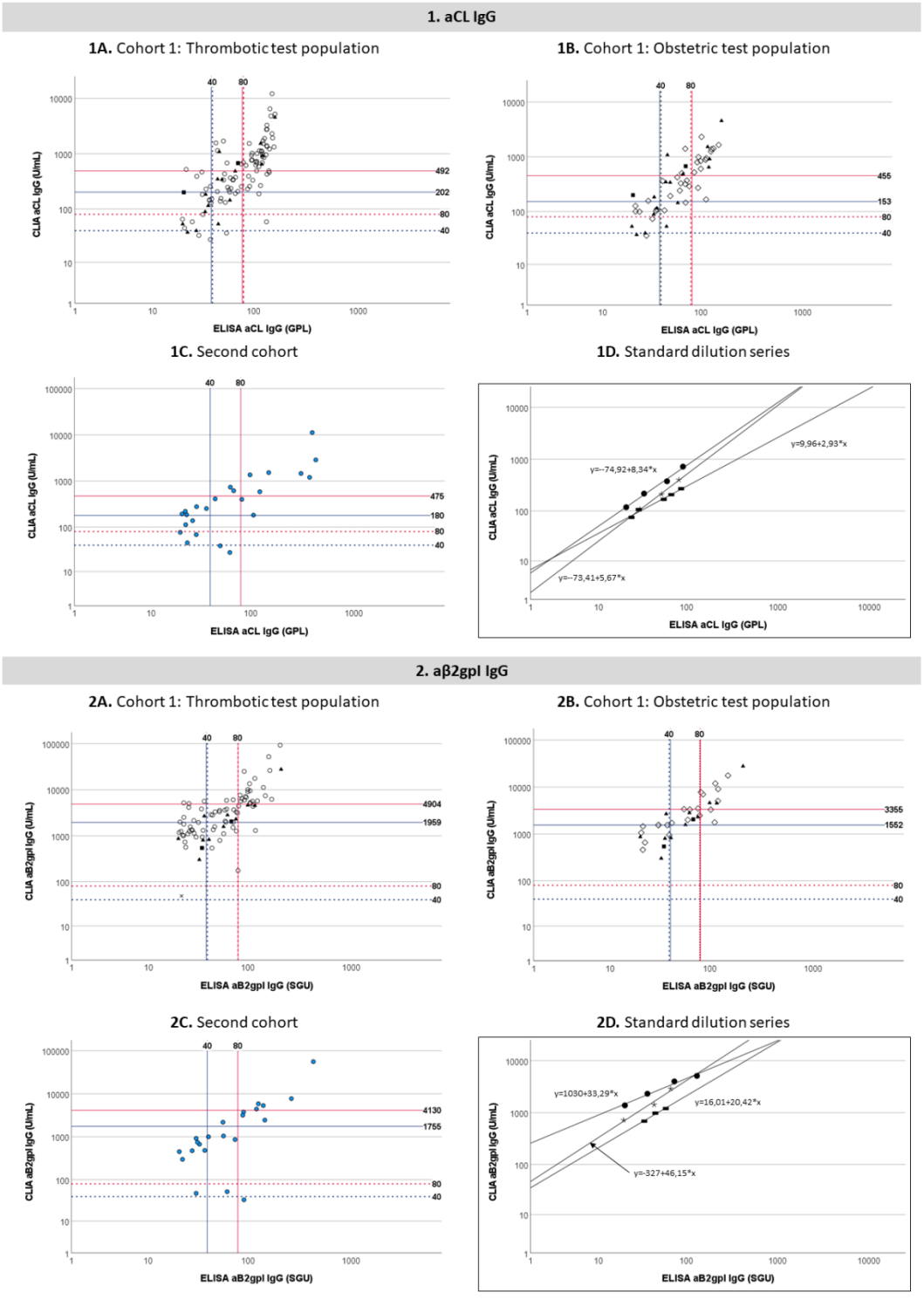
ROC signifies the method for determining ranges with ROC based sensitivity for CLIA, *Sapporo*, *Harris, and EM6* signify the method for ranges determination based on linear regression with the respective standards for CLIA. LR+ denotes the positive likelihood ratio for diagnosis of thrombotic APS and obstetric APS in the thrombotic test population and obstetric test population, respectively.

FIGURE 1 CLIA compared to ELISA for IgG isotype aCL and aβ2gpI.

Graphic representation of aCL IgG (1) and aβ2GPI IgG (2) with thrombotic test population (A), obstetric test population (B), second cohort (C) and reference standard series (D). (A) and (B): Clear circles: thrombotic APS, clear squares: obstetric APS, filled squares: HC, filled triangles: AID, cross: non-APS thrombosis. (A), (B) and (C): vertical and horizontal lines, blue: moderate threshold, red: high threshold, dotted lines: 40-80 units thresholds, solid lines: ROC sensitivity-based thresholds. (D) Sapporo, Harris standards and EM6 monoclonal antibodies measurements with corresponding regression lines (filled circles, rectangles, asterisks, respectively), vertical lines: moderate and high threshold values for ELISA (40 and 80 'units') and horizontal lines: corresponding threshold titres for CLIA.

FIGURE 2 CLIA compared to ELISA for IgM isotype aCL and aβ2gpI.

Graphic representation of aCL IgM (1) and aβ2GPI IgM (2) with thrombotic test population (A), obstetric test population (B), second cohort (C) and reference standard series (D). (A) and (B): Clear circles: thrombotic APS, clear squares: obstetric APS, filled squares: HC, filled triangles: AID, cross: non-APS thrombosis. (A), (B) and (C): vertical and horizontal lines, blue: moderate threshold, red: high threshold, dotted lines: 40-80 units thresholds, solid lines: ROC sensitivity-based thresholds. (D) Sapporo and Harris standards with corresponding regression lines (filled circles and rectangles, respectively), vertical lines: moderate and high threshold values for ELISA (40 and 80 'units') and horizontal lines: corresponding threshold titres for CLIA



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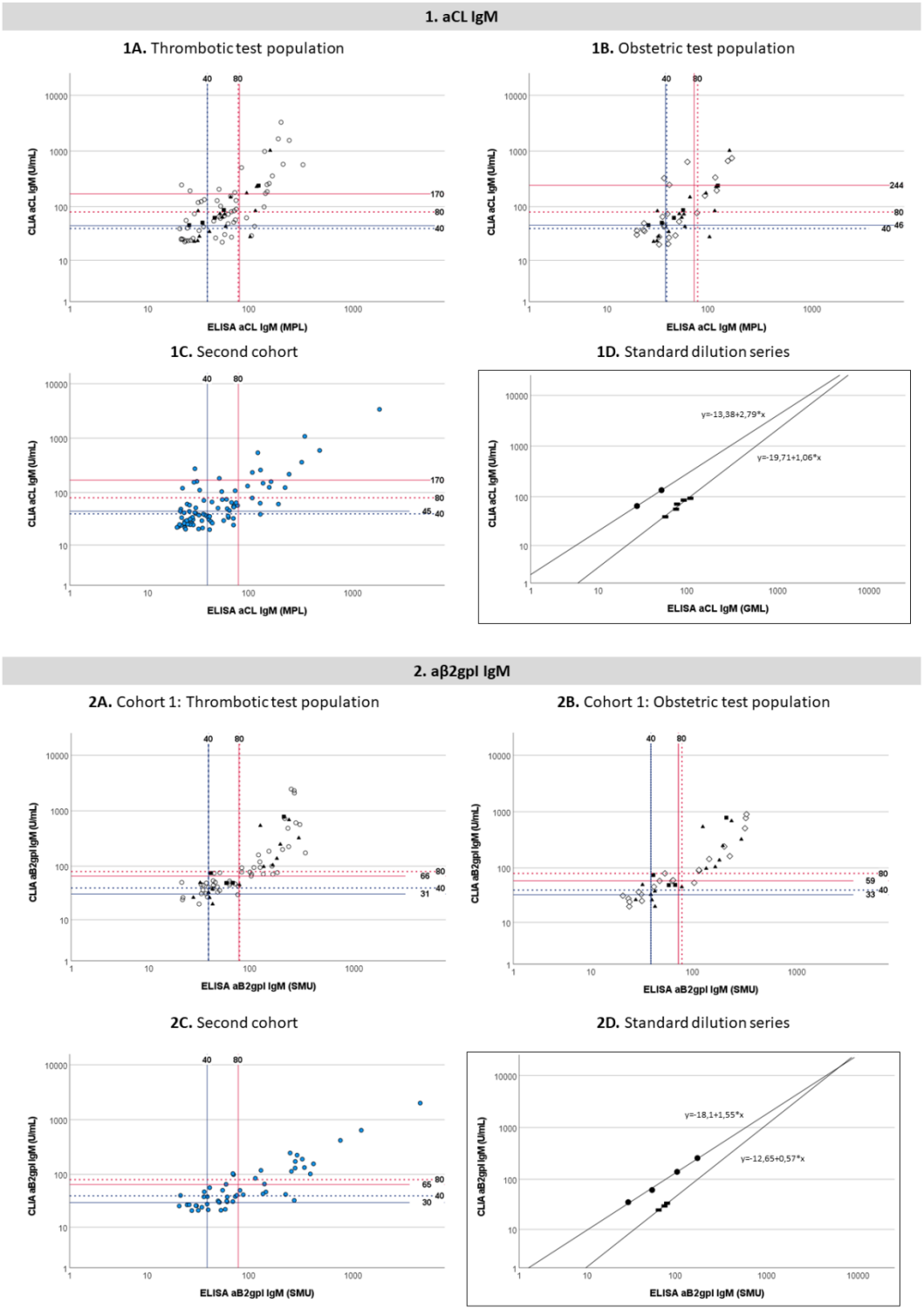

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