Received: 9 October 2017 | Accepted: 30 November 2017

DOI: 10.1002/rth2.12071

BRIEF REPORT

The impact of repeated freeze-thaw cycles on antiphospholipid antibody titer

Karel Maelegheer MD | Katrien M. J. Devreese MD, PhD

Coagulation Laboratory, Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, Ghent, Belgium

Correspondence
Katrien Devreese, Coagulation Laboratory, Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, Ghent, Belgium.
Email: katrien.devreese@uzgent.be

Abstract

Background: Pre-analytical factors, like freeze-thaw cycles (FTC), can potentially affect results and clinical interpretation. According to the SSC-ISTH recommendations for antiphospholipid antibodies (aPL) testing, additional FTC should be avoided to maintain the best performance. Patient samples are often analyzed in batch and having one frozen sample aliquot for all aPL tests, that may hamper daily routine work-out. To use them for study or method validation purpose, sample storage in bio banks is often done in one aliquot also triggering the need for several FTC to be able to use them in different scientific projects. Taking into account the limited scientific literature, the strict guidelines and the potential benefits of repeated FTC we evaluated this pre-analytical factor.

Objectives: Evaluating the effect of repeated FTC on anticardiolipin (aCL) IgM/IgG and anti-beta-2 glycoprotein 1 (aβ2GPI) IgM/IgG antibody titer.

Patient/Methods: 42 patient plasmas that were not thawed before, were retrieved from the routine archive (−80°C). All aliquots were analyzed on five consecutive days with an additional, standardized FTC every day. Mann-Withney tests for statistical differences and a concordance correlation coefficient (CCC) were calculated between the first and following FTC.

Results and Conclusion: For all four aPL no statistical difference or degradation from positive to negative was seen, even after five FTC. The CCC between the first and fifth FTC were between 0.98 and 1 for all four aPL. aCL IgM/IgG and aβ2GPI IgM/IgG antibody titer, over a broad titer range, are stable over time and after repeated FTC.

KEYWORDS

antibodies, anticardiolipin, antiphospholipid antibodies, beta 2-Glycoprotein I, diagnostic tests, protein stability, routine

Essentials

- Limited data are available of freeze-thaw effect on anticardiolipin and anti-beta2-glycoprotein I antibodies.
- Patient samples with antibody titer over a broad range were analyzed in a standardized freezing-thawing scheme.
- aCL and aβ2GPI IgG and IgM titers are stable over time and after repeated freeze-thawing.
- Repeated use of samples facilitates routine work-out and use of samples for scientific purposes.

1 | INTRODUCTION

Beside clinical symptoms, the diagnosis of antiphospholipid syndrome (APS) is based on the presence of persistent antiphospholipid antibodies (aPL). The three main cornerstone antibodies are anticardiolipin (aCL) IgM/IgG, anti-beta-2 glycoprotein 1 antibodies (aβ2GPI) IgM/IgG, and lupus anticoagulant (LAC). Among many challenges in aPL measurement, the pre-analytical conditions may influence test results.[1,2] Following the Standardization subcommittee (SSC) of the international society of thrombosis and haemostasis (ISTH) recommendations, FTC should be avoided for all aPL.[3,4] In contrast to the effect of freeze-thawing in phospholipid-dependent clotting assays used for LAC, there is little scientific literature for solid phase assays (SPA).[5] However, APL testing is mainly performed in batch due to the potential negative influence of pre-analytical variables, such as repeated FTC, on test results.

Evaluating the effect of repeated FTC on aCL IgM/IgG and aβ2GPI IgM/IgG could generate practical benefits for routine testing. Another useful application could be the use of stored samples for research and method evaluations.

2 | METHODS

Patient plasmas (n = 42) were selected based on routine results to cover a wide titer range for all four APL, and on availability of an unthawed aliquot (Table 1).

Citrated plasma was frozen after double centrifugation.[3] Samples were collected between 2012 and 2017. First analysis of each included sample was performed on aliquot not thawed before. Further, samples were analyzed on five consecutive days with an additional, standardized FTC every day (Figure 1). All analysis were performed by an automated chemiluminescent assay (HemosIL AcuStar, Instrumentation Laboratory-Werfen, Bedford, MA, USA) using the same reagent lot for each parameter during the study.

Statistical analysis was done in Medcalc (MedCalc Statistical Software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium). A Mann-Withney test for statistical differences ($P < .05$) between the first and following FTC was performed. If no statistical difference was obtained after five FTCs, a concordance correlation coefficient (CCC) was calculated.

TABLE 1 Routine results for all selected patient samples (n = 42) stored in the routine archive

	Negative		Around Cut off		Positive		Strong positive	
	Range (U/ml)	# samples	Range (U/mL)	# samples	Range (U/ml)	# samples	Range (U/mL)	# samples
aCL IgG	3-12	8	21-34	6	53-54	3	103-480	10
aCL IgM	2-18	7	22-27	4	52-70	7	104-2330	6
aβ2GPI IgM	3-9	7	21-48	8	54-88	3	107-2705	6
aβ2GPI IgG	11-18	9	23-97	12	119-186	4	213-3153	12

aCL, anticardiolipin; aβ2GPI, anti-beta 2 glycoprotein I.

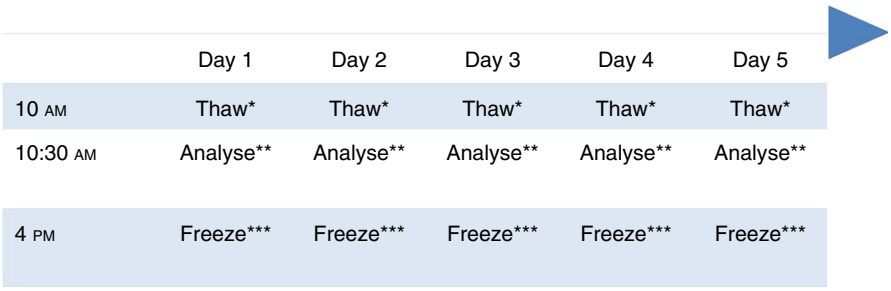

FIGURE 1 Timelaps studydesign
*Thawing (37°C water bath 5 minutes);
**Start analysis (every day in same order);
***Freezing of all samples at −20°C

TABLE 2 Overview of Mann-Withney test results between the first and following freeze thaw cycle

	Samples	FTC1 med(IQR)	FTC2 med (IQR)	*P*-value (FTC1-2)	FTC3 med (IQR)	*P*-value (FTC1-3)	FTC4 med (IQR)	*P*-value (FTC1-4)	FTC5 med (IQR)	*P*-value (FTC1-5)
aCL IgG	26	30(8-93)	32 (13-263)	.8692	33(13-274)	.7418	35(147-237)	.7349	34(13-276)	.7143
aCL IgM	24	55(13-87)	57(11-88)	.8528	58(11-90)	.765	59(11-86)	.7259	57(8-76)	.8481
aβ2GPI IgM	25	31(8-93)	28(9-98)	.8234	31(8-99)	.6908	29(9-102)	.7784	31(8-81)	.7949
aβ2GPI IgG	37	74(22-676)	73(18-724)	.9354	79(15-694)	.8642	74(11-748)	.9058	74(13-723)	.9551

aCL, anticardiolipin; aβ2GPI, anti-beta 2 glycoprotein I.

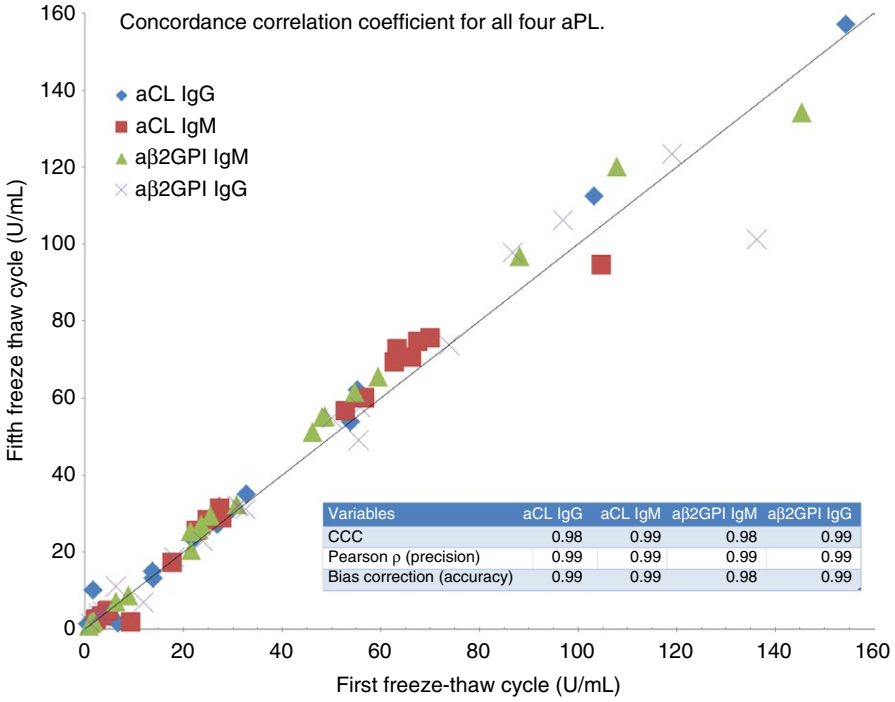

FIGURE 2 The CCC calculated between first and fifth FTC are graphically shown. Values above 200 U/mL are disregarded in the graph (for visual purposes), but were included in the concordance correlation coefficient calculation and showed no significant difference with values below 200 IU/ml aCL, anticardiolipin; aβ2GPI, anti-beta-2 glycoprotein 1; CCC, concordance correlation coefficient; FTC, freeze-thaw cycle

3 | RESULTS AND DISCUSSION

To exclude long term storage as a potential source of degradation, all aPL titers after one FTC were compared with the original results (initially also obtained after one FTC). No significant differences between these results were observed. An interesting feature of these results, knowing that some of these samples were already stored for 5 years, is the stability over time. Having a closer look at samples stored frozen for more than 2 years, no significant difference was seen between the initial results and the results after one FTC in the current study. The sample results, 8 for aCL IgG, aCL IgM aβ2GPI IgM, and 13 for aβ2GPI IgG, showed an excellent correlation coefficient (0.94-0.99) between the initial results and the result after one FTC obtained in this study. Due to the limited number of samples stored more than three years, no useful statistical analysis could be performed to confirm an even longer stability.

After five FTC, none of the samples degraded from positive to negative and no false positive results were seen. Also, no significant titer changes (defined as > 10%) were seen after five FTC. Between the first and following repeated FTCs a Mann-Withney test was performed for all aPL to look for statistical significant differences in titer (Table 2). Even after five FTC none of the aPL tested gave a significant difference in results. A correlation between the titers was calculated and illustrated in Figure 2, showing graphically and mathematical, the good correlation for all four aPL with a CCC between 0.98 and 1.

Considering the high CCC, we could exclude a significant inter-run variability as potential influencing factor on variability of titers. For each sample, a coefficient of variation (CV) was calculated using the test result after each FTC. Comparing the median CV value (for each aPL divided in four categories as described in Table 1) with results obtained from quality control (QC) material no significant difference was found. All median sample CV values varied between 4 and 15.6% comparing to the QC CV values of these runs, between 2.3-13.9%. These results confirm the stable character of the antibodies and the limited effect of FTC.

Furthermore, 4 months later, a selection of these samples with sufficient sample volume, were additionally thawed and analyzed using another batch of reagent. No significant differences were observed between first FTC and these results. As a final test, these samples were also analyzed on a second analyzer of the same type, also resulting in non-significant differences. Correlating results after the first FTC on analyzer 1 and results after the sixth FTC on a second analyzer a concordance correlation coefficient was found, depending on the antibody, between 0.91-0.98. Comparing both analyzers, with a sample size varying between 13 and 23 samples, an excellent correlation coefficient of 0.99 for each antibody was found, confirming the robustness of this automated systems.[6]

aPL are very heterogeneous group of antibodies. The small sample size, although covering a broad titer range, may be seen as a limitation of this study. Also, all tests were performed using one and the same analytical platform. Other test systems are known to be less robust suffering from lab to lab variation or high between-run variation.[6,7] Using this automated system excludes these variables, making it possible to exclusively evaluate the effect of freeze-thaw cycles.

4 | CONCLUSION

We evaluated the stability of aPL titers after several FTC. aCL and aβ2GPI IgG and IgM, over a broad titer range including samples around the cut-off value, are stable over time and after repeated FTC. With this study, we excluded this pre-analytical factor as source for variability in aPL titer measured by chemiluminescent assays.

RELATIONSHIP DISCLOSURE

No conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

KMJ Devreese conceived the study idea and supervised, consulted, and corrected the study design, statistical analysis and manuscript. KR Maelegheer developed the study design, performed practical and statistical analysis, and wrote the manuscript.

ORCID

Karel Maelegheer http://orcid.org/0000-0003-0774-2595
Katrien M. J. Devreese http://orcid.org/0000-0002-7559-2579

REFERENCES

1. Devreesez K, Hoylaerts MF. Laboratory diagnosis of the antiphospholipid syndrome: a plethora of obstacles to overcome. Eur J Haematol. 2009;83:1–16.
2. Devreese KM. Antiphospholipid antibody testing and standardization. Int J Lab Hematol. 2014;36:352–63.
3. 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. J Thromb Haemost. 2014;12:792–5.
4. Pengo V, Tripodi A, Reber G, et al. Update of the guidelines for lupus anticoagulant detection. Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. J Thromb Haemost. 2009;7:1737–40.
5. Brey RL, Cote SA, McGlasson DL, Triplett DA, Barna LK. Effects of repeated freeze-thaw cycles on anticardiolipin antibody immunoreactivity. Am J Clin Pathol. 1994;102:586–8.
6. 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. J Thromb Haemost. 2017;15:91–5.
7. 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. Semin Thromb Hemost. 2012;38:390–403.

How to cite this article: Maelegheer K, Devreese KMJ. The impact of repeated freeze-thaw cycles on antiphospholipid antibody titer. *Res Pract Thromb Haemost*. 2018;2:366–369. https://doi.org/10.1002/rth2.12071