Storage in ultra-low-temperature decreases the levels of IgM anticardiolipin antibody in serum samples from tuberculosis patients

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***Abstract*:** The evaluation of novel tuberculosis (TB) biomarkers relies on analysis of previously stored sample sets. We aimed to determine the effect of storage temperature on serum IgM anticardiolipin antibody levels in samples from TB patients. Ultra-low-temperature storage decreased IgM anticardiolipin levels. We recommend against using ultra-low-temperature storage when investigating IgM anticardiolipin biomarker-based tests.

Keywords: Biomarker, IgM anti-cardiolipin, tuberculosis, storage temperature, therapy monitoring

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Biomarkers are used to determine whether patients are responding successfully to pharmacological tuberculosis (TB) treatment. Serum IgM antiphospholipid antibodies have been proposed as biomarkers for monitoring TB therapy [Goodridge *et al.* 2012; Wallis *et al.* 2013]. We previously demonstrated that IgM anticardiolipin (anti-CL) antibody levels decrease after two months of treatment. The aim of our study was to determine the effect of temperature and storage conditions on the integrity of serum IgM anti-CL antibodies [Pierangeli and Harris, 2008].

Serum samples were collected from 16 patients diagnosed with pulmonary TB from Colon City, Panama. TB diagnosis was achieved either by acid fast bacilli sputum smear or sputum culture. None of the patients had been previously diagnosed with TB. The average age of the patients was 34 years old (range 23–46), and 10 patients (62.5%) were men. Only one patient was coinfected with human immunodeficiency virus (HIV). Each of the 16 serum samples was divided into seven subsets: three subsets were stored for 1 week on crushed ice, at −20°C or at −80°C respectively; three subsets were stored for 1 week at the same temperatures, but the samples were exposed to five freeze–thaw cycles (FTCs); and one subset was maintained at 4°C for 4 weeks [Nassta *et al.* 2013]. The levels of serum IgM anti-CL antibodies were determined for each sample using a modification of a previously described in-house enzyme-linked immunosorbent assay (ELISA) [Goodridge *et al.* 2012]. Differences between the 4°C sample subset and the other six subsets were determined by the Wilcoxon signed rank test and considered significant for p values less than 0.05. A serial dilution of a whole human IgM fraction was analyzed using the in-house ELISA to prepare a standard curve. Each dilution was measured in triplicate. A linear regression was obtained for the six optical density values of each IgM dilution. The standard curve resulted in an $R^2 = 0.9908$ ($p < 0.0001$).

We compared the serum IgM anti-CL antibody levels between the sample subsets stored at different temperatures for 1 week. The serum IgM anti-CL antibody levels in the sample subset stored for 1 week on ice and at −20°C were not significantly different from those of the sample subset stored at 4°C ($p > 0.05$). The sample subset stored for 1 week on ice showed IgM anti-CL antibody levels that were nearly the same as the levels in the sample subset stored at 4°C. However, the sample subset stored for 1 week at −20°C showed a non-significant 46% decrease in serum IgM anti-CL levels when compared with the sample subset maintained at 4°C. The sample subset stored at −80°C for 1 week displayed a significant 55% decrease in serum IgM anti-CL levels ($p = 0.012$) when compared with the sample subset maintained at 4°C (Figure 1).

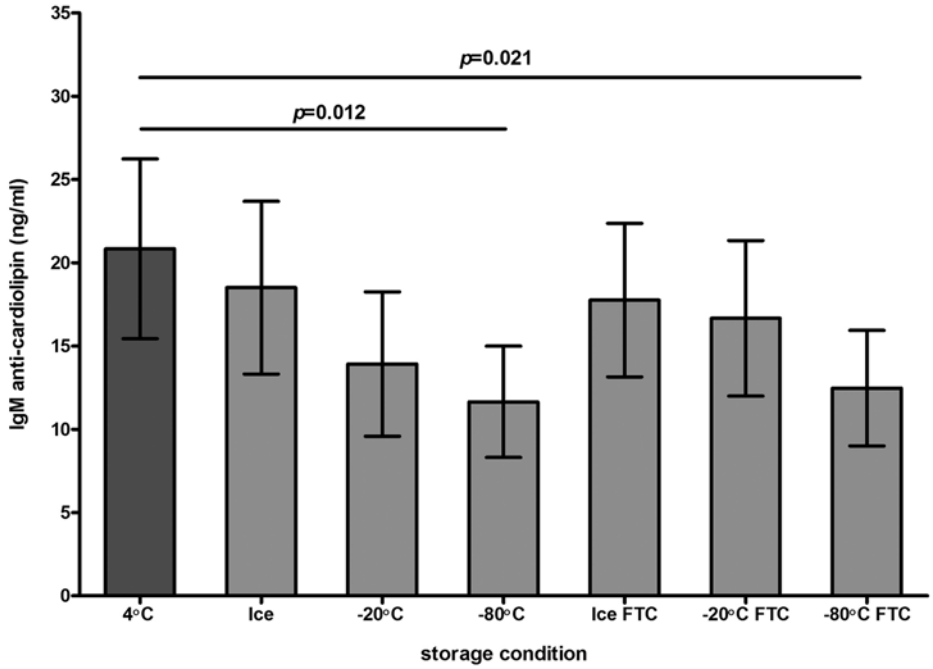

Figure 1. IgM anticardiolipin antibody levels under different storage conditions. A total of 16 serum samples from pulmonary tuberculosis patients were prepared and divided into 7 subsets. Three serum sample subsets were stored for 1 week on crushed ice, at –20°C or at –80°C, respectively. Similarly, three serum sample subsets were stored under freeze–thaw cycles (FTC) over 1 week on crushed ice, at –20°C or at –80°C. Another sample subset was stored at 4°C. IgM anticardiolipin levels were determined by enzyme-linked immunosorbent assay. Arrows indicate the standard error. The Wilcoxon signed rank test was used to compare levels between the sample subsets. Significant differences are indicated with the corresponding *p* values.

The FTC did not alter serum IgM anti-CL levels. The sample subset that underwent FTC over 1 week at −20°C showed a nonsignificant, 36% decrease ($p > 0.05$) in IgM anti-CL antibody levels when compared with the sample subset stored at 4°C. However, significant differences in serum IgM anti-CL antibody levels were observed between the sample subset stored at 4°C and the sample subset stored under FTC at −80°C ($p = 0.021$). Similarly, the serum IgM anti-CL antibody levels of the sample subset stored at −80°C significantly decreased by 52% ($p = 0.012$) when compared with the sample subset stored at 4°C (Figure 1). We also evaluated storage for 4 weeks at 4°C *versus* −20°C. We found a nonsignificant decrease in serum IgM anti-CL in the sample subset stored at −20°C compared with the sample subset stored at 4°C (data not shown).

A previous study reported a similar effect of temperature on IgM, IgG and IgA anti-CL [Brey *et al.* 1994]. The authors added known concentrations of freeze-dried polyclonal human immunoglobulins to serum samples from healthy donors. Although this study revealed a significant decrease in IgG but not in IgM or IgA anti-CL antibodies, the study procedure does not resemble actual sample handling for biomarker tests during TB therapy monitoring. In contrast, our study showed that samples from TB patients can be stored on crushed ice, at 4°C or −20°C or under FTC for 1 week without serum IgM anti-CL undergoing significant degradation. Similarly, these samples can be stored at −20°C for a period of 4 weeks without significant degradation. We found that serum IgM anti-CL stored at −80°C, either at a constant temperature for 1 week or under FTC, experienced degradation, presumably by cryoprecipitation. Proteins precipitate out of solution when frozen plasma is thawed and refrozen at very low temperatures [O'Shaughnessy *et al.* 2004]. In our study, we observed a white precipitate in samples that were stored at both −20°C and −80°C. A detailed study is warranted to determine the effect of ultra-low-temperature storage on IgM anti-CL and other biomarker proteins across different TB patient care and therapy schemes. Such studies should evaluate total IgM, IgM anti-CL and IgM against purified protein derivative (PPD) before, during and after storing

serum samples from TB patients. This approach will clarify whether IgM antibodies should be measured immediately after obtaining serum samples. Similar evaluations of antibody biomarkers for monitoring treatment of other chronic infections and autoimmune diseases, such as the 16/6 idiotype for systemic lupus erythematosus, are also recommended [Blank and Shoenfeld, 2008]. For now, we recommend against using sample sets stored at ultra-low temperatures when investigating serum IgM anti-CL antibody biomarker-based tests in TB patients.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

References

Blank, M. and Shoenfeld, Y. (2008) The story of the 16/6 idiotype and systemic lupus erythematosus. *Isr Med Assoc J* 10: 37–39.

Brey, R., Cote, S., McGlasson, D., Triplett, D. and Barna, L. (1994) Effects of repeated freeze–thaw cycles on anticardiolipin antibody immunoreactivity. *Am J Clin Pathol* 102: 586–588.

Goodridge, A., Cueva, C., Lahiff, M., Muzanye, G., Johnson, J., Nahid, P. *et al.* (2012) Anti-phospholipid antibody levels as biomarker for monitoring tuberculosis treatment response. *Tuberculosis (Edinb)* 92: 243–247.

Nassta, G., Prankerd, R. and Mcintosh, M. (2013) Effect of freezing on oxytocin ampules. *N Engl J Med* 368: 2145–2146.

O'Shaughnessy, D., Atterbury, C., Bolton Maggs, P., Murphy, M., Thomas, D., Yates, S. *et al.* (2004) Guidelines for the use of fresh-frozen plasma, cryoprecipitate and cryosupernatant. *Br J Haematol* 126: 11–28.

Pierangeli, S. and Harris, E. (2008) A protocol for determination of anticardiolipin antibodies by ELISA. *Nat Protoc* 3: 840–848.

Wallis, R., Kim, P., Cole, S., Hanna, D., Andrade, B., Maeurer, M. *et al.* (2013) Tuberculosis biomarkers discovery: developments, needs, and challenges. *Lancet Infect Dis* 13: 362–372.