CVI Accepts, published online ahead of print on 12 March 2008
Clin. Vaccine Immunol. doi:10.1128/CVI.00012-08

Prevention of assay interference in the LIAISON® infectious serology tests

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Immunoassay interference causing unexpected reactive results in magnetic microparticle based assays was detected. A systematic evaluation of LIAISON® EBV IgM showed that 5% of the positive results (0.4% of tested samples) could be explained by such interference. Adding chemical blocking reagents (polyvinylpyrrolidone and polyvinyl alcohol) to the assay buffers partially prevented this phenomenon.

The LIAISON® diagnostic system (DiaSorin, Saluggia, Italy) is a convenient, automated immunoassay platform based on chemiluminescence and antigen/antibody coated magnetic microparticles (4, 8). Immunoassay interference was suspected when, in a patient suffering from chronic fatigue, positive results were found for *Borrelia burgdorferi* sensu lato immunoglobulin M (IgM) (index: 7.0; cut-off: 1.1), cytomegalovirus IgM (70 mU/L; cut-off: 30 mU/L), Epstein-Barr virus (EBV) IgM (1620 mU/L; cut-off: 40 mU/L) and herpes simplex virus IgM (index: 32; cut-off: 1.1). None of these positive results could be confirmed, neither with other immunoassays nor with immunoblotting. The patient serum contained neither rheumatoid factor nor paraprotein. We further thoroughly investigated the serum from this patient, we estimated the frequency of this type of interference and we demonstrate a simple and inexpensive solution to partially prevent the problem.

In the experimental methods described below, appropriate positive and negative control samples were used to detect any undesirable effects of the procedures.

The index sample was pretreated with RF-Absorbent (Dade Behring, Marburg, Germany), which contains sheep IgM antibodies targeted against human IgG-Fc fragments: 250 µL of RF-Absorbent was added to 250 µL of serum, briefly vortexed and incubated 1 h at room temperature. This procedure, which precipitates IgG along with rheumatoid factor, did not affect the results and excludes an insufficient sample pretreatment in the LIAISON® assays.

Heterophilic antibody interference was excluded by treating the sample, according to the manufacturer's instructions, with a non specific antibody blocking tube (Scantibodies Laboratory, Santee, CA, USA). We also treated the sample by adding 40 µg of PolyMAK-33 (MAK33-

IgG1/IgG1 Poly, Roche Diagnostics, Mannheim, Germany) to 250 µL of serum and incubated this mixture 1 h at room temperature. PolyMAK 33 is a polymerized murine IgG1 preparation, superior in blocking heterophilic antibody activity compared to polyclonal mouse immunoglobulins (9). This procedure did not affect the results.

However, incubating 250 µL of sample with 75 µL unlabeled beads (kindly provided by Diasorin) at room temperature for 15 min and centrifuging this mixture for 5 min at 2000 x *g*, completely eliminated the interference. Apparently IgM antibodies from the patient reacted with the solid phase in the assays. To test whether this reactivity was restricted to one specific type of microparticle, we evaluated seven different types of microparticles (Dynabeads, Dynal Biotech, Oslo, Norway): M-270 amine, M-270 carboxylic acid, M-270 epoxy, M-280 sheep anti-mouse IgG, M-270 streptavidin, M-280 streptavidin, and M-280 tosylactivated. 250 µL of serum was added to approximately 0.4 x 10^9 beads, briefly vortexed, and incubated 15 min at room temperature. After centrifugation (5 min, 2000 x *g*), the supernatant was analyzed. The interference could be completely eliminated using M-280 tosylactivated beads or M-270 epoxy beads and partially eliminated when using M-270 amine beads, M-280 streptavidin beads or M-280 sheep anti-mouse IgG beads. No effect was seen incubating the serum with M-270 streptavidin beads nor with M-270 carboxylic acid beads.

From these experiments we concluded that, (a) since the M-270 streptavidin beads are based on carboxylic acid beads, the described interference would probably not occur in assays applying carboxylic acid based beads. (b) The interference could be completely eliminated with unlabeled tosyl activated beads but only partially with tosyl activated beads already covered with an antigen

(M-280 streptavidin**,** M-280 sheep anti-mouse IgG). Increasing antigen density on the beads might prevent contact of IgM with the bead surface, avoiding the bead-linked interference. However, increasing the antigen density is not easily achieved, additional chemical blocking could be an alternative solution for the problem. To test this hypothesis, polyvinylpyrrolidone (PVP-360, Sigma-Aldrich) and polyvinyl alcohol (P8136, Sigma-Aldrich) was added to the sample diluents used in the assays (11, 12). This modification indeed strongly reduced or completely eliminated the interference effect. Polyvinylpyrrolidone and polyvinyl alcohol, both water-soluble polymers, can minimize background signals mainly by competing with nonspecific adsorption of proteins to the solid phase (2, 6, 10).

Since interference was most prominent in the EBV IgM assay, we focused on this assay and found that adding polyvinylpyrrolidone and polyvinyl alcohol to the EBV IgM dilution buffer (buffer A) at a final concentration of 0.1 % and 0.005 % respectively, was optimal in reducing the interference. Although this procedure did not completely abolish the strong interference in the index patient, the effect was significantly reduced: the EBV IgM concentration fell from 1620 mU/L in the original assay to 320 mU/L in the modified assay. This easy, inexpensive modification allowed us to estimate the frequency this interference occurs, by using this modified assay on 120 consecutive samples with a positive result for EBV IgM (> 40 mU/L). Results from this comparative study are shown in figure 1 as a Bland-Altman plot. Discrepant results were defined as differing more than 24 % (three times the inter-assay coefficient of variation) between the original and modified assay. Six discrepant results were found (5% of EBV IgM positive samples; 0.4 % of all EBV IgM requests). Interpreting the results as suggested by the

manufacturer (< 20 mU/L = negative; 20 – 39 mU/L = equivocal; ≥ 40 mU/L = positive), one sample became negative and three became equivocal by using this modified assay. Determination of EBV IgM on these six samples using an enzyme-linked immunosorbent assay (Enzygnost Anti-EBV/IgM II, Dade Behring) and an immunoblot (Euroline anti-EBV-profile 2, Euroimmun, Lübeck, Germany) showed them to be EBV IgM negative. These six samples were also positive for herpes simplex virus IgM on LIAISON® (index range: 1.9 – 2.8) but negative using an enzyme-linked immunosorbent assay (Enzygnost Anti-HSV/IgM, Dade Behring). One sample was also positive on LIAISON® for *B. burgdorferi* sensu lato IgM (index: 1.5) but negative on immunoblot (*Borrelia afzelii*-westernblot, Euroimmun).

Immunoassay interference through endogenous antibodies such as rheumatoid factor or heterophilic antibodies remains a continuing challenge (1, 3, 7). The interference we described here was apparently caused by the direct binding of IgM antibodies to surface modified polystyrene microparticles. These 'solid phase reactive antibodies' have also been described in flow cytometry based multiplex bead array assays (5, 12). In this technology, the use of reagent blank beads can detect high background signals caused by polyreactive antibodies or non-specific binding antibodies. This important advantage is however not present in the LIAISON® and many other immunoassay platforms. Since this type of assay interference cannot be predicted nor easily recognized in the LIAISON®, the inexpensive preventive measure we propose can reduce the number of false positive results.

We are grateful to the Diasorin Belgian customer support team for providing us with necessary information on LIAISON® assay applications and for providing unlabeled beads we used in our initial experiments. We also thank A. Vereecken and G. Salembier for their support to this study.

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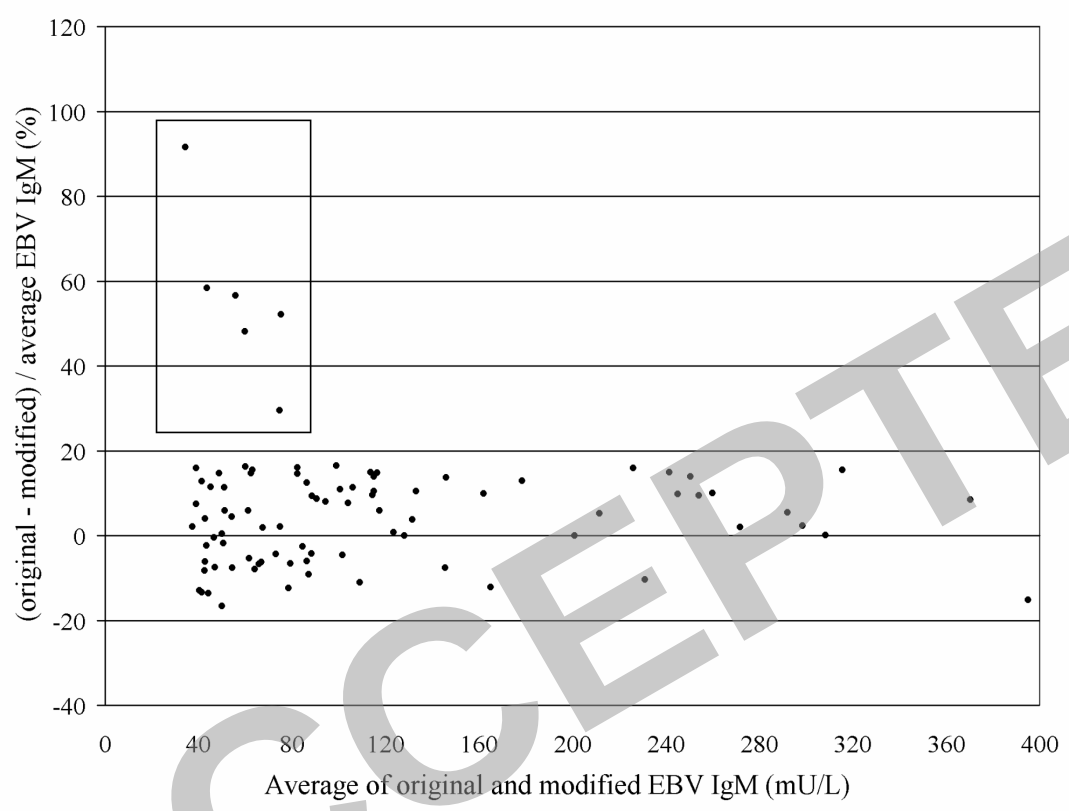
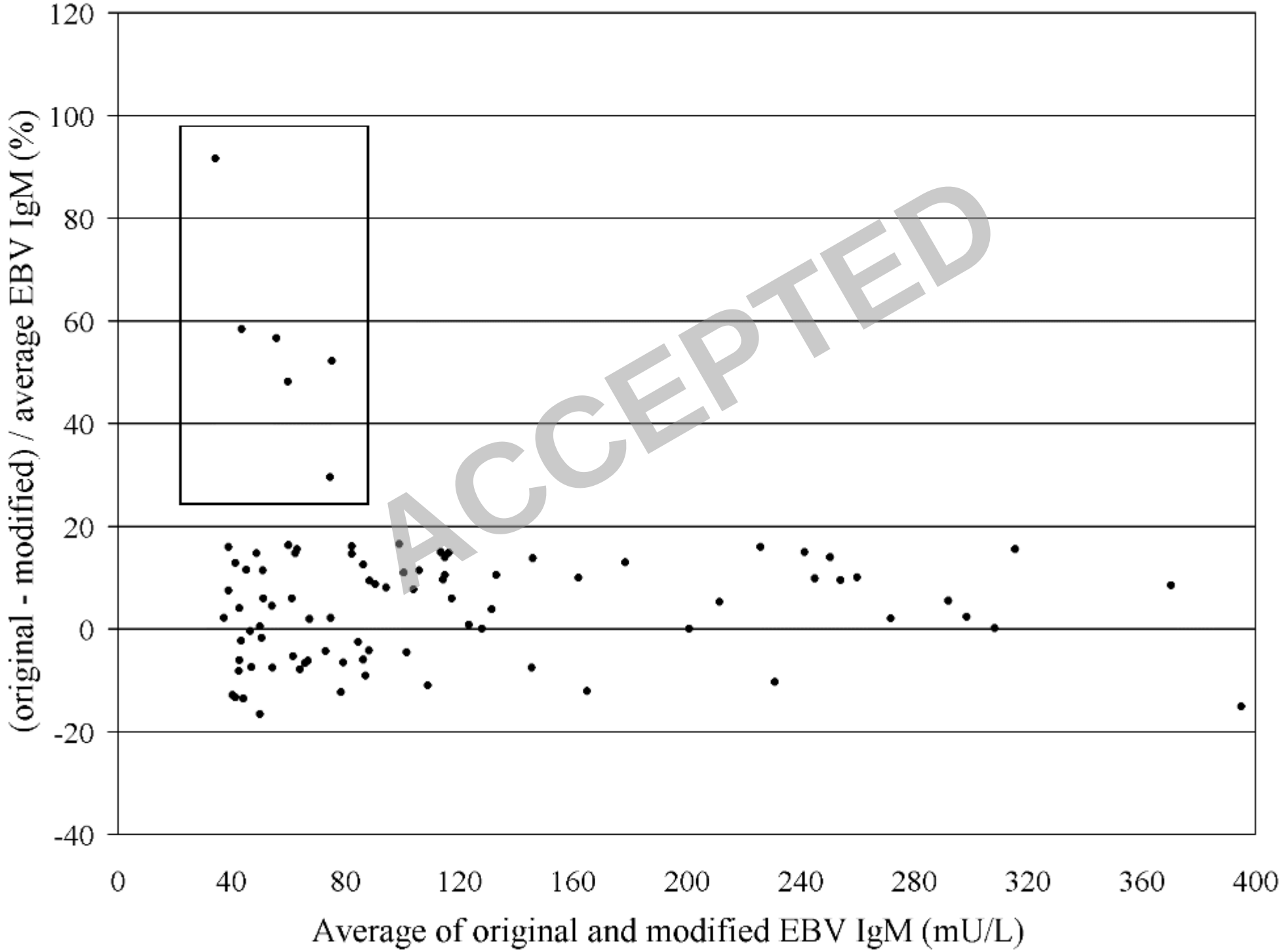


Figure 1. Bland-Altman plot comparing results obtained with the modified and original EBV IgM assay on LIAISON®. The six discrepant results are marked (box). Only results < 400 mU/L are shown (n = 89).


120
100
80
60
40
20
0
-20
-40
(original - modified) / average EBV IgM (%)
0
40
80
120
160
200
240
280
320
360
400
Average of original and modified EBV IgM (mU/L)