

1 Prevention of assay interference in the LIAISON[®] infectious serology tests

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1 **Immunoassay interference causing unexpected reactive results in magnetic microparticle**
2 **based assays was detected. A systematic evaluation of LIAISON[®] EBV IgM showed that**
3 **5% of the positive results (0.4% of tested samples) could be explained by such interference.**
4 **Adding chemical blocking reagents (polyvinylpyrrolidone and polyvinyl alcohol) to the**
5 **assay buffers partially prevented this phenomenon.**

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

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

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

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

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

5 However, incubating 250 μ L of sample with 75 μ L unlabeled beads (kindly provided by
6 Diasorin) at room temperature for 15 min and centrifuging this mixture for 5 min at 2000 \times g,
7 completely eliminated the interference. Apparently IgM antibodies from the patient reacted with
8 the solid phase in the assays. To test whether this reactivity was restricted to one specific type of
9 microparticle, we evaluated seven different types of microparticles (Dynabeads, Dynal Biotech,
10 Oslo, Norway): M-270 amine, M-270 carboxylic acid, M-270 epoxy, M-280 sheep anti-mouse
11 IgG, M-270 streptavidin, M-280 streptavidin, and M-280 tosylactivated. 250 μ L of serum was
12 added to approximately 0.4×10^9 beads, briefly vortexed, and incubated 15 min at room
13 temperature. After centrifugation (5 min, 2000 \times g), the supernatant was analyzed. The
14 interference could be completely eliminated using M-280 tosylactivated beads or M-270 epoxy
15 beads and partially eliminated when using M-270 amine beads, M-280 streptavidin beads or M-
16 280 sheep anti-mouse IgG beads. No effect was seen incubating the serum with M-270
17 streptavidin beads nor with M-270 carboxylic acid beads.

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

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

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

1 manufacturer (< 20 mU/L = negative; $20 - 39$ mU/L = equivocal; ≥ 40 mU/L = positive), one
2 sample became negative and three became equivocal by using this modified assay.

3 Determination of EBV IgM on these six samples using an enzyme-linked immunosorbent assay
4 (Enzygnost Anti-EBV/IgM II, Dade Behring) and an immunoblot (Euroline anti-EBV-profile 2,
5 Euroimmun, Lübeck, Germany) showed them to be EBV IgM negative. These six samples were
6 also positive for herpes simplex virus IgM on LIAISON[®] (index range: 1.9 – 2.8) but negative
7 using an enzyme-linked immunosorbent assay (Enzygnost Anti-HSV/IgM, Dade Behring). One
8 sample was also positive on LIAISON[®] for *B. burgdorferi* sensu lato IgM (index: 1.5) but
9 negative on immunoblot (*Borrelia afzelii*-westernblot, Euroimmun).

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

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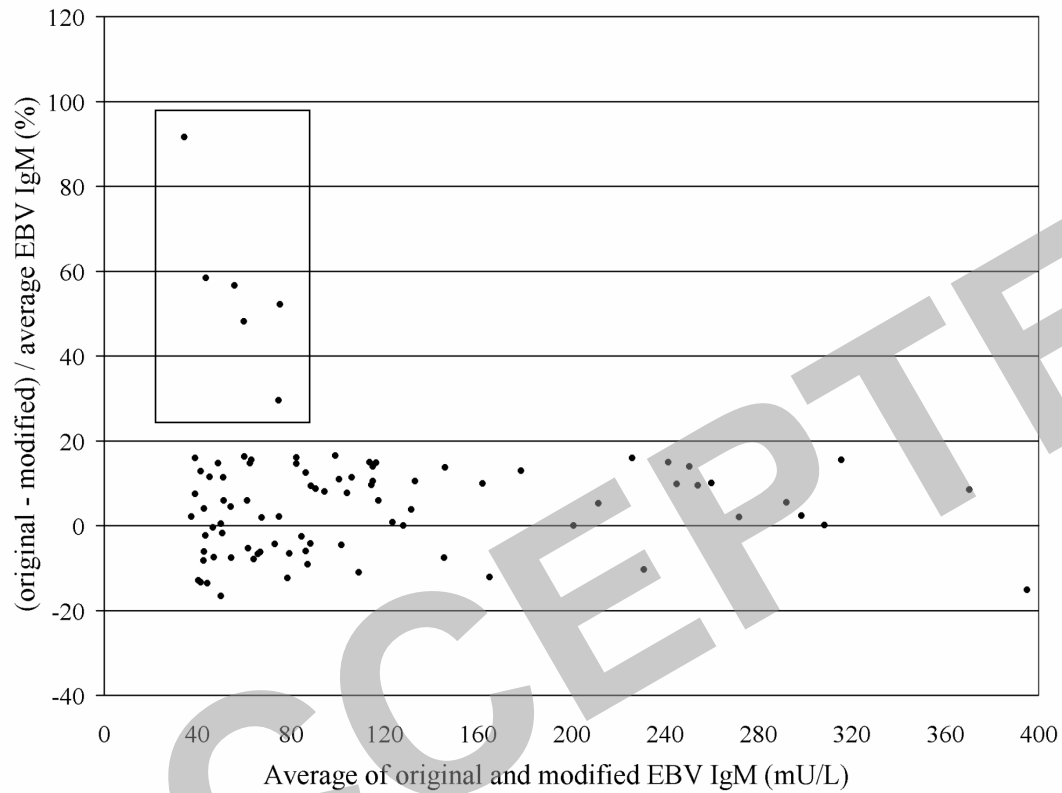
1 We are grateful to the Diasorin Belgian customer support team for providing us with necessary
2 information on LIAISON[®] assay applications and for providing unlabeled beads we used in our initial
3 experiments. We also thank A. Vereecken and G. Salembier for their support to this study.

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

