Author's response to reviews

Title: Utilization of serology for the diagnosis of suspected Lyme borreliosis in Denmark: Survey of patients seen in general practice

Authors:

Ram B Dessau (ramd@regionsjaelland.dk)
Jette M Bangsborg (jeban@heh.regionh.dk)
Tove Ejlertsen (tove.ejlertsen@rn.dk)
Sigurdur Skarphedinson (d238406@dadlnet.dk)
Henrik C Schønheyder (hcs@rn.dk)

Version: 4 Date: 17 October 2010

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A new version of the manuscript has been prepared and changes have been carefully marked:
  additions with blue+underscore
  deleted passages with red+strike-through

Author's response to reviews: see over
**Reviewer’s report**

**Title:** Utilization of serology for the diagnosis of suspected Lyme borreliosis in Denmark: Survey of patients seen in general practice  

**Version:** 3  
**Date:** 24 September 2010  
**Reviewer:** Pascal Meylan

**Reviewer’s report:**
I believe that the controversy between the authors and reviewer 2 is rooted in the misunderstanding that this study is a study of serology utilization, not of serology performance, in the diagnosis of Lyme borreliosis.

We thank the reviewer for this clarification.

On one hand, the authors are clear about that study goal (see title or the background section). On the other hand, they express more than once opinions suggesting that the single tier approach used in Denmark (though not the International Standard) is performing quite well, based on blood donor data (see page 3 laboratory methods).

We are aware that the Danish approach, also used to some extent in Sweden is not the same as the German MIQ or USA standard, therefore a part of the discussion is devoted to inform the reader about IDEIA assay used. The IDEIA assay has has also been used by others in a number of publications (e.g. 1-5) including comparisons with other assays. However, an extensive consideration of this issue is beyond the scope of the current study.

In fact, based on personal experience, I have major doubts for instance on the specificity of flagellin based IgM ELISAs, based on IB data in particular.

Could this experience be based on the recombinant p41/internal fragment? In our laboratory we have experience with the Mikrogen Recomblot. In this IB the p41 bands are present in many samples tested, in both IgM and IgG, and this includes many samples where the IDEIA flagella assay is negative, also in Danish blooddonors. The in vitro reactivity of the IDEIA native flagella antigen is not necessarily comparable to p41 antigens implemented in other assays?

But if one looks at the present study data, is it likely that regarding an infection that leaves long lasting IgG blood levels (unless treated early) IgM seroprevalence would be 3 fold that of IgG? Unless the majority of infections are caught and treated at an early time (which may be true for patients seeking care, but not for a population), leaving a majority of patients with transient IgM responses only, the alternate hypothesis that flagellin IgM specificity in patient population is much lower than in blood donor sounds a lot more likely.

The authors agree that the total rates of seropositivity do not reflect whether patients are positive due to active infection with Borrelia or nonspecific reactivity in excess of the healthy adult donorpopulation. It may be a combination of both and the relative contribution is unknown.

The large discrepancy between IgM and IgG seropositivity is indeed an interesting and unexplained finding. We do not agree that this may be explained by non specific IgM responses only.

A sentence has been added on page 6 at the beginning of the paragraph about these hypotheses and the limitations in this study and a new paragraph has been added starting in the last paragraph on page 9.
I note also that a good deal of the discussion ("seropositivity rates and the clinical variables) is devoted to discussing how the OR of having specific IgG or IgM are related to the clinical presentation, providing a kind of post hoc validation of the tests.

The point that we intend to make is that the OR of having specific IgG or IgM are related to the clinical presentation and the selection of patients for testing and is not as such a validation of the test method. The OR’s and the observed differences are central to the study and therefore we consider the discussion of possible interpretations and limitations to be relevant.

As an example, see page 9 second paragraph. I think that it is close to be abusive to try to deduce a test specificity from its use in this patient population (suspected Lyme arthitis), assuming that at that stage all patients should be IgM negative.

The authors do not agree on this point. By using the phrase “….maximum number of false positives…..” we intended to make a distinction from “all”. To clarify this further we have added “lowest estimate” to the last sentence in the paragraph in this new version of the manuscript. Accordingly the full sentence now reads: This implies that the lowest estimate of the specificity for the IgM antibody test could be 94.6 percent instead of the expected 98%.

In the end, the limits of the present paper re test performance, as acknowledged by the authors, lie in the lack of gold standard (short of detailed clinical and serological characterization, or PCR or culture documentation of infection), which can only be performed in (non representative) subpopulations.

The authors agree fully.

Of note also, there is no mention of the fact that IgM result interpretation should be very cautious in those patients with more than 4-8 weeks of illness.

This is discussed on page 8 – second paragraph. See also the other additions to the manuscript indicated above which also discuss this topic.

Still, I find a lot of value in this paper that, in quite original manner, present the use and limits of current Lyme testing in large population.

We thank the reviewer for this comment.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: I declare that I have no competing interests
Reference List


