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

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**Version:** 3  **Date:** 14 July 2010

**Author's response to reviews:** see over
To the Editor, BMC Infectious Diseases Journal.

The data presented in the present manuscript concerns the use of Borrelia serology and possible problems in patient selection. We find it important that these unique data become published and available not only to those with closely related research interests and but also to others who are engaged in diagnosing Borrelia infections on a daily basis. We agree with the reviewer (HG), that the data presented in this study is open to different interpretations regarding the relative importance of patient selection and test characteristics (sensitivity and specificity).

The reviewer (HG) doubts the quality of the flagella ELISA, especially the sensitivity of the IgG assay. This is a relevant concern. Which test strategy to use for Borrelia serology is an ongoing debate (1,3). The flagella ELISA has been used on the Scandinavian Market for about 20 years and was in 2008 used by 17 laboratories in Denmark, Norway and Sweden (unpublished data presented at (1), and only in three of these as part of a two-tier strategy. This test is thus widely accepted in Scandinavia. The clinical experience does not indicate serious problems with sensitivity or technical failures of the test. The sensitivity and specificity of flagella ELISA is in our opinion quite well documented in peer reviewed published studies, both in the older original studies by the developer and in more recent comparisons with other assays. It is not reasonable to indicate that the “test is not usefull”. It is of course possible that the sensitivity may be lower in a consecutive stream of routine patients where also more atypical or more early cases are included, compared to the evaluation studies based on “well defined” cases. But this may be a relevant problem for any other Borrelia-test as well.

The critique presented by the reviewer is not related to any shortcomings of the study as such, but a pointer to further investigations and a discussion of the results. It is exactly the intention of the study to provide data for such a discussion.

Changes in the manuscript:
Page 5: A reference has been cited to document the specificity in a large Danish blood donor population instead of a “personal communication” (no 5).
Page 6: correction to 2% (1.6% deleted) to correspond to the expected specificity of 98% cited in the methods section.
Page 8 top: Correction of typing error.
Page 8 bottom: A comment on the possibility of a lower sensitivity for IgG has been added.

Changes to the references:
Reference no 5 added as indicated above.
Reference to the recently published EUCALB case definition has been added (no. 19).
Reference to the EUCALB website has instead been deleted.

All changes have been carefully marked:
additions with blue+underscore
deleted passages with red+strike-through
On the following pages 3-5, detailed responses to the comments of reviewer 1 (HG) are added directly in the review.

We kindly ask the Editor to consider the disagreement between the conclusion of reviewer 1 (HG) and reviewer 2 (MP).

The reasons why we chose to submit our manuscript to BMC Infectious Diseases was not only because of the open access, but also because we appreciate the policy of BMC to publish the prepublication history on the internet, as this very relevant discussion may contribute to the readers’ understanding of the complexity of this observational study.

Yours Sincerely,

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Detailed point by point response:

Reviewer’s report
Title: Utilization of serology for the diagnosis of suspected Lyme borreliosis in Denmark: Survey of patients seen in general practice
Version: 2 Date: 27 April 2010
Reviewer: Hermann Girschick

Reviewer’s report:

Major compulsory REmision:

After the Revision the critical questions of the last comments of mine have not been addressed in detail. I do appreciate that in the literature the Flagellin ELISA test has a high sensitivity of 98% for late Borreliosis. However, if the clinical pictures reported are somewhat rightly recognized (Acrodermatitis, Erythema migrans) then, the results of the tests are unexplained even in an Area of low background serology.

We agree with the reviewer that the data presented in this manuscript raises a lot of questions concerning the routine use of Borrelia serology. That is the purpose of the study.

Any positive result of serology may be due to active disease, old immunity or cross reactivity. Any negative result may be truly so or a false negative.
Laboratory errors are rare.

We agree with the reviewer that the relative distribution of these six different interpretations is not known and are unexplained by the study. However this study is an observational study of the clinical routine and should be seen as a supplement to studies on carefully preselected samples. Therefore logistic regression has been used to show the relative differences in the rates of Borrelia serology with less emphasis on the absolute rates of seropositivity.

Erythema migrans (EM) is the most common manifestation of Lyme Borreliosis. The majority of classical and obvious cases are commonly treated based on clinical diagnosis alone. Probably the cases selected for serological testing are patients with a less characteristic rash and the clinician is in doubt. Assuming a sensitivity of ~50% and ~20% IgM or IgG positive (table 1.) then ~40% of these must be true EM cases. A pretest probalility of roughly 40% in this group is in our opinion “somewhat rightly recognized”.

Acrodermatitis (ACA) is much more rare and only 67 patients were tested on this suspicion. 10.4% were IgG positive. Because of the low number of observations the confidence interval for the odds-ratio estimate was wide (1.24-8.25). In our experience, a common differential diagnosis is discolouring of the skin due to venous insufficiency of the lower leg, indeed a very common disease. We do not know the incidence of ACA in Denmark as the disease is not notified. Our data indicate with correction for the response rate an incidence of about 12 in 1.5 million inhabitants corresponding to about 44 in the total 5.5 million inhabitants in Denmark. Given the rarity of the diagnosis a pretest probability of about 10% is also “somewhat rightly recognized”. This may be compared to an average of 80 officially reported cases of neuroborreliosis per year in Denmark, which are probably also about 50% underreported.
Therefore a control serology using western blotting or others ELISAs really would help to understand what had happened in this study.

We disagree. There is no gold standard or well defined “control serology”. When doing more tests more positive results become available. Doing more tests may increase the risk of false positive results more than the sensitivity which is already relatively high. For a discussion of this issue, please see the attached article (2). Adjustment of the ELISA results with a control Immunoblot/ELISA would yield fewer positive results. Detailed recommendations on how to design these double assays are not available. Which test to use first? How to adjust cutoffs when combining tests? How to choose the scoring of the many possible combinations of bands on the immunoblot is not well-documented.

We are working on how to design combinations of tests to gain sensitivity without losing the specificity (2).

From a clinical standpoint the test is not useful with such low positive results in obviously classical Borrelia diseases.

We disagree that the test is not useful. As stated in the manuscript: “It must also be expected that some patients with vague clinical symptoms are tested to rule out the diagnosis of LB rather than to confirm it.” In this situation a high specificity is of importance.

We still believe the sensitivity of the flagella assay (IgM or IgG) is adequate and comparable to other ELISAs on the market. Please see the references cited in the manuscript and in (2).

Higher levels of seropositivity could be found using other tests. As part of recent routine in-house evaluations we have examined different commercial kits. These ELISA kits have a common problem of a higher rate of seropositivity of IgM or IgG in samples from healthy Danish blood donors (C6 immunetix 10%, Mikrogen RecomWell 18%, Siemens Enzygnost 7%) compared to 4.7% for the flagella ELISA (2). It has been chosen not to implement these tests, as they have a poor specificity in a Danish background population, while the sensitivity of the flagella ELISA was only slightly lower compared to the other tests.

The model of logistic regression has been chosen to examine the relative yield of positive results according to clinical manifestations. When choosing another test with a higher rate of positive results (not reflecting active Borrelia infection) the tendency could be to get lower and less significant odds-ratios, even if the sensitivity is higher.

Page 8: A comment on the possibility of a lower sensitivity for IgG has been added.

If the authors do not dare to test all patients a significant subgroup testing would help themselves to understand more in detail, how a diagnosis fits to the test and how the test fits to the (obviously often questionable) diagnosis

We agree that evaluation of classifier performance of Borrelia tests is needed. The reason such studies are difficult to perform is that a gold standard test for LB is not available. However this is outside the scope of the present study.
There is much concern about Lyme Borreliosis, including misconceptions of chronic Lyme disease (3), and in the daily clinical experience of the authors the low rate of seropositivity fits to the (obviously often questionable) diagnosis. In our opinion some overuse of Borrelia serology is the most likely explanation for the low rates of IgG seropositivity.

In short, we have very little information from the published literature, from the in house comparisons and from our practical clinical experience to indicate the IgG flagella ELISA has a sensitivity inferior to other similar tests.

Please also read the recent publication (2) from APMIS included with this response, where an improvement of cut-off calculation is proposed.

**Level of interest:** An article whose findings are important to those with closely related research interests

*Thank you for this comment*

**Quality of written English:** Acceptable

*Thank you for this comment*

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

*Thank you for this comment*

**Declaration of competing interests:**
I declare that I have no competing interests' below.

**References:**
2. Dessau et al. APMIS 2010; 118: 313–323