Reviewer's report

Title: Detection of human leptospirosis as a cause of acute fever by capture ELISA using a Leptospira interrogans serovar Copenhageni (M20) derived antigen.

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Reviewer: Eva Ruzic-Sabljic

Reviewer's report:

General comments:
Authors reported about new serological ELISA test for diagnosing leptospirosis; they wished to assess the sensitivity, specificity and predictive value of an IgM antibody capture enzyme linked immunoassay (MAC-ELISA) derived from the M20 strain of Leptospira interrogans serovar Copenhageni. Regarding laboratory diagnosis of leptospirosis, authors compared results of the new test with MAT, which was used as the gold standard method for diagnosis. Although authors designed their study properly, some deficiencies of the study have to be completed. First of all, it is confused which group of patient were used and for which testing? We suggest to present particular patient’s group with which authors achieved particular result (sensitivity, specificity, leptospirosis confirmation, etc).

Title page
The title indicates well the study design.
Title page should indicate corresponding author that I miss in sent the article.

Abstract has 299 words that fulfill criteria for abstracts (up to 350 words), and is well structured into separate sections. Methods and Results are not reported well. Please, check the following comments.

The Background is clearly stated. The section ends with a brief aim that is being reported in the article. Some printing errors must be corrected, and I suggest authors to check carefully the text. This section also lacks reference no. 10, please add it.

Methods
1. Herein it is not clear which patients groups were included and tested with both IgM MAC-ELISA and MAT?
In the first part of the section authors report “…. patients aged five years or more with fever equal to or greater than 38.0° C for equal to or less than 7 days who presented for medical care …….. were invited to participate”; in the second part of the section authors report: “Patients were ineligible to participate if a clinically identifiable focus of infection was present”. But later on in this section (Assessment for MAC-ELISA assay cross-reactivity with other pathogens)
authors report that “Banked sera specimens from patients with evidence of other infections were also used as negative controls” and “were tested by the leptospirosis MAC-ELISA assay to assess for cross-reactivity between leptospirosis and these other pathogens”. So, if patients with determined infections were included for IgM MAC-ELISA validation, authors must state their number as well as number of patients suffering by particular infection different than leptospirosis.

Moreover, in the section “Determination of MAC-ELISA optical density cut-off» authors report about “50 banked Leptospira-negative control sera from healthy subjects from Lima, Peru and 18 banked positive control sera samples from Peruvian patients with known MAT-confirmed”.

Taking together, a lot of participants were tested with IgM MAC-ELISA? So, the section Material and Methods must be supplemented with exactly cited patients’ groups:

- Febrile illness of unknown etiological agents
- Febrile illness caused by brucellosis, bartonellosis, yellow fever, dengue, Oropouche virus, Caraparo virus, Venezuelan Equine Virus, P. vivax, P. falciparum, hepatitis A, hepatitis B, HIV, and Treponema pallidum
- 50 banked Leptospira-negative control sera
- 18 banked positive control sera samples

2. It is not clear how authors determined results of IgM MAC-ELISA?

In the section “Determination of MAC-ELISA optical density cut-off« authors cited two groups of patients (50 banked Leptospira-negative control sera from healthy subjects from Lima, Peru and 18 banked positive control sera samples from Peruvian patients with known MAT-confirmed leptospirosis) to calculate OD cut-off but later (in Results) they did not report any data of these two patients groups? At the same time authors reported (section Sample testing by IgM MAC-ELISA:) »Positivity of acute and convalescent sera was defined as a reactive IgM at titers of # 1:400«. So, it is not clear how authors determined results of IgM MAC-ELISA, by OD or by titer? These parts must be written clearly.

3. In section “Sample testing by MAT:« authors did not clarify which group of patients was tested? All four groups: 1. Febrile illness of unknown etiological agents; 2. Febrile illness caused by other pathogens, 3. 50 banked Leptospira-negative control sera; and 4. 18 banked positive control sera samples?

4. In section “Sample testing by MAT:« L. borgpetersenii is written incorrectly (borgEpetersenii). Please, correct.

Results

In this section, results of testing of all four groups of patients must be reported. Herein, it is not clear where authors find 63 participants and from which group
were they? Section Results lacks raw results of all testing. The same is for Table 1, is not correct because it is not known which patients are these 63 reported here?

Sera were tested in acute and convalescent phase. Can authors report results of particular testing? Did authors find any difference between acute and convalescent results? Can authors report these findings?

Authors cannot report sensitivity, specificity, PPV, and NPV without previously reporting raw results of all testing, and detailed calculation of particular subject. The same is for Table 2. In this part of Results, the HIV positive patient appeared. So, results of testing of all four patient groups are necessary.

Tables
Tables must be without abbreviations (MAC, MAT, M20, CI, etc)

Discussion
Regarding previously comments for Material and Methods and Results, Discussion can be valuated after cited all missing data.

It is not clear how many patients with febrile illness were not diagnosed as leptospirosis, and rested negative either by MAT and IgM MAC-ELISA?

References
References are not cited well (comma, bold/italic letters, “et all”, etc). I suggest authors to look at Instruction for authors.

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

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' below.