Author's response to reviews

Title: Evaluation of seven serological assays for diagnosis of tularemia

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Author's response to reviews: see over
Dear Miss Ramos,

Thank you for giving us the opportunity to submit a revised version of our manuscript entitled “Evaluation of seven serological assays for diagnosis of tularemia” to BMC Infectious Diseases (MS: 5811156010473437).

We changed the title to “Performance of seven serological assays for the diagnosis of tularemia” and added one author who performed statistical analyses that were suggested by the reviewers. The work was subject to STARD guidelines and we assessed the diagnostic value of all tests.

Attached you will find:

- Our point-by-point response to the concerns of the reviewers.
- Our revised manuscript in which we have addressed the reviewers’ and editors’ comments.
- Supplementary data with all test results.

We hope our revisions sufficiently address the referees’ concerns and our revised version is now acceptable for publication.

Yours sincerely,

Herbert Tomaso

Reviewer 1

Major Compulsory Revisions

Information of tularemia patients and their sera would be necessary to describe. How were they diagnosed as tularemia? By serological method (which method?), PCR or isolation of bacteria? Which clinical period were the sera taken?
In accordance with WHO guidelines, patients with typical symptoms were regarded as tularemia cases, if at least one serum sample was positive in the microagglutination assay (MAT) for tularemia. If a single serum was negative, paired serum samples were tested after two weeks or later.

**Cut-off value for in-house ELISA should be determined by ROC and two-graph-ROC curve analyses.**

We have used ROC curves created with MedCalc to determine the cut-off value for the in-house ELISA as suggested by the reviewer.

**Minor Essential Revisions**

1. 77

   The authors described ‘novel assays’. However, it is unclear which assays are novel methods in this paragraph. The authors should indicate clearly.

   We deleted “novel”. We added further details in Material and Methods.

1. 123 **ATCC29648 is not Francisella tularensis.**

   We corrected this typographical mistake: ATCC 29684

1. 212 According to table 1, the sensitivity of the in-house ELISA was 79%.

   **Table 1 What are meaning ‘Positive’ and ‘Negative’ in column ‘n’? Tularemia patients and Healthy donors?**

   We have completely revised the dataset and accordingly also Table 1 in order to follow the other reviewer’s suggestions and corrected the text. All data should be consistent now.

**Reviewer 2:**

**Major Compulsory Revisions**

1. The authors have to explain why they have not used more sera for calculating specificity.
In the present study, the number of sera of tularemia cases is higher than in most existing studies, but it is true that it would be desirable to test more true negative sera from a non-endemic region (which is the southern hemisphere). We mention this limitation and the consequences in the discussion now.

2. It has to be discussed why the results of those positive sera with a volume not sufficient to be tested in all tests have been used for calculation of sensitivity. It is advisable to exclude these sera from the calculations at all or to improve the table by giving the full set of data for the sake of transparency.

As suggested we excluded all sera that were not sufficient for all assays.

3. The authors have to explain why they included obviously couples of sera from patients for calculation of sensitivity.

We included paired sera, because seroconversion is time-dependent and assays react differently. A supplement with all raw data will be provided.

Minor essential revisions

line 40: please explain 'dangerous to handle'.

This statement was deleted because it was redundant (“highly infectious...”).

line 45: remove sentence 'nine patients.....'

The sentence was deleted.

3. line 90: the sentence should read 'in nine Serbian patients other diseases were diagnosed.....' The information 'tularemia was not confirmed' is misleading.

The paragraph was rewritten.
4. line 153: explain why a dilution of 1:100 was used.

A dilution of sera reduced the non-specific background observed in control sera and increased the test efficiency. This phenomenon was earlier described in the following study:


5. line 164: explain what 'characterised sera' means.

The sentence was corrected: “positive and negative control sera”

6. line 205: explain the difference between sensitivity and diagnostic sensitivity.

This was a meaningless duplication in the text, which we corrected. However, all definitions of parameters that were used to determine the diagnostic value are now provided in the text:

The diagnostic value was determined based on the following parameters: Diagnostic sensitivity = $\frac{TP}{(TP+FN)} \times 100$; Diagnostic specificity = $\frac{TN}{(TN+FP)} \times 100$; Positive Predictive Value (PPV) = $\frac{TP}{(TP+FP)} \times 100$; Negative Predictive Value (NPV) = $\frac{TN}{(TN+FN)} \times 100$; Diagnostic accuracy = $\frac{(TP+TN)}{(TP+TN+FP+FN)} \times 100$.

7. line 211: remove (especially early in the course of infection). This assumption is not proved by the data shown.

The sentence was deleted.

8. line 223: explain 'diagnostic sensitivity'.

We use only the term diagnostic sensitivity to be consistent as described above.

9. line 240: explain why this test is preferable when testing sera 'early after infection'.

This statement was deleted.
10: line 250: this sentence is not correct.

This statement was deleted.