Peer Review File

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Review Comments-reviewer A

1) First, the title needs to indicate the other focus of this study, the diagnostic accuracy for PCa.

Reply 1) We have corrected the title to "Consistency and diagnostic accuracy of 4 assays in the detection of the total and free prostate-specific antigen" and the running title to "Consistency and diagnostic accuracy of 4 assays for total and free PSA"

Changes in the text: We have modified our text as advised. Please see Page 1, line 3-4, line 20.

2) Second, the abstract needs further revisions. The background did not indicate the clinical significance of this research focus and what has been known on the consistency between the four methods in western populations. The methods need to describe the inclusion of subjects, and statistical methods for assessing the measurement consistency and the diagnostic accuracy. The results need to summarize the clinical characteristics of the study sample and report the diagnostic accuracy parameters. The conclusion should not repeat the main findings, which should have comments for the clinical implications of the findings.

Reply 2) We have modified the abstract as advised. 2.1 For the background, we corrected to "The lack of interchangeability among prostate-specific antigen (PSA) assays causes difficulties in clinical interpretation. " and this is the clinical significance of this research focus on. For Mindray CL6000I chemiluminescence system used in this study is a Chinese platform, there is no data in western populations, there is no consistency evaluation between the four methods in western populations. 2.2 For the methods, we have added more information about the inclusion of subjects, and statistical methods. 2.3 For the results, we have refined them.2.4 For the conclusion, we have refined and added more information.

Changes in the text: Please see Page 2, line 3-4, line 9-14, line 16-17, line 19-21; Page 3, line 4-5.

3) Third, in the introduction of the main text, the authors need to analyze the potential reasons for the variations in the measurement values of different methods for PSA and clearly indicate the clinical question to be answered by the consistency analysis. The authors need to review the literature on the consistency across the four test methods in western populations.

Reply 3) 3.1 We added more information for the potential reason for the variations in the measurement values of different methods for PSA and added one more reference(number 5). . The potential reason is " The main reasons for assay variability were the non-equimolar detection of tPSA and the non-uniform assay calibration" . 3.2 For Mindray CL6000I chemiluminescence system used in this study is a Chinese platform, therefore, no western populations are tentatively involved, There was no consistency assessment among the four methods in Western populations.

But there was an evaluation of consistency among the three methods. References 8-11 are consistency assessments of mainstream testing in Western populations. We also revised the sequence of some content.

Changes in the text: Please see Page 3, line 14-15, line 32-33; Page 4, line 13-17, line 23-26.

4) Fourth, the methodology of the main text needs to describe the clinical research design, sample size estimation, the data collection of the clinical characteristics of the subjects, the diagnosis of PCa, and quality control measure for ensuring the test accuracy of the four methods. In statistics, please ensure P<0.05 is two-sided.

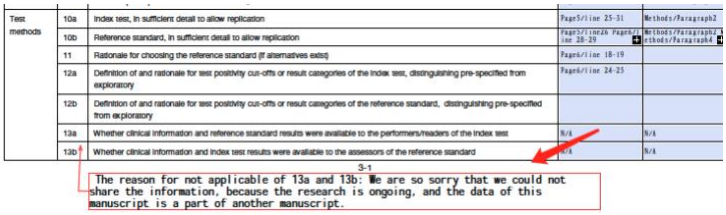
Reply 4) We have modified the methodology of the main text as advised. 4.1 For clinical research design: this is a methodological comparison study using the residual serum. All of the four methods used the same samples. After eligible consecutive patients enrolled, the residual serum would be divided into 4 aliquots and tested by 4 different assays. Then the values of tPSA and fPSA were compared with the Beckman assays as the reference standard. The accuracy of diagnosis of the 4 assays are also compared with the pathological findings as the reference standard. These information were described in "Subjects", "Blood sample collection and assays" and "Methodological comparisons". 4.2 For sample size estimation, According to EP-9A3, simple size of methodological comparison is at least 120, and 30 is minimum simple size required by the statistics, we completed samples enrollment when the sample size of prostate cancer is about 30. These information were described in "Subjects". 4.3 We added more information about collection of the clinical characteristics of the subjects, the diagnosis of PCa, and quality control measure for ensuring the test accuracy of the four methods. 4.4 We have confirmed P<0.05 is two-sided, and corrected to " A two-sided P value of <0.05 was considered statistically significant".

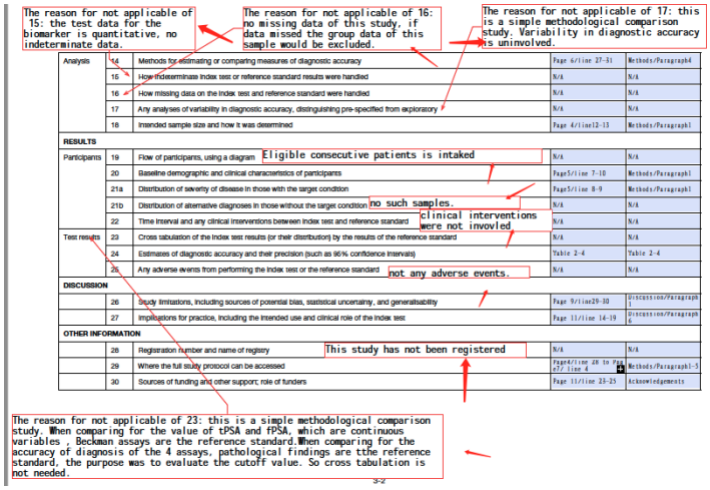
Changes in the text: Please see Page 5, line 7-10, line33-34; Page 6, line 1-2, line 6-11, line 15, line 22-24, line 27-31. Page 7, line 4.

Review Comments-reviewer B

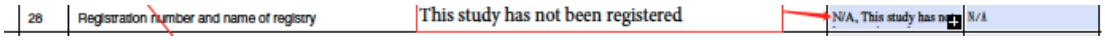
1. STARD checklist:

a. Please directly put those reasons in the non-applicable items respectively.





Take this item for example:



b. Item 29: We could not find such information in your paper, please check if you've prepared a study protocol to be shared; If not, please fill "N/A" in this item.

Reply 1. Thanks, we have modified the STARD checklist as advised.

2. Running title is limited to 60 characters. Please shorten the running title which should be within 60 characters including spaces.

Reply 2. We have shorten the running title to "Consistency and diagnostic accuracy of 4 assays for PSA", and there are 55 characters including spaces.

Please see the changes in Page 1, line 21.

3. Please structure your Main Text as: **Introduction, Methods, Results, Discussion, Conclusion**. Please add "Conclusion" section for your manuscript.

Reply 4. We have added the "Conclusion" section.

Please see the changes in Page 11, line 15.