Reviewer's report

Title: Modified Enzyme Multiplied Immunoassay Technique of Methotrexate Assay to Improve Sensitivity and Reduce Cost

Version: 0 Date: 24 Aug 2018

Reviewer: Reviewer 2

Reviewer's report:

PEER REVIEWER COMMENTS: To view the full report from the academic peer reviewer, please see the attached file.

REVIEWER COMMENTS FROM REPORT: Overall this research focuses on increasing the sensitivity of methotrexate assay which is important for the management of treatment and prognosis in various diseases. The authors have decreased the volume of the assay reagent to improve the lower limit of quantification and hence making it cost effective as well. The study was done in clinical samples. Decreasing the volume of the reagent can increase the sensitivity of the lower limit of quantification but it might compromise the ability of upper limit of quantification of the kit. I would highly recommend that the authors should do another set of experiments to report the range of this assay to identify whether the range of lower and upper limit of detection improves or one is compromised, whereas the other is improved. This can be done on a serial dilution of Mtx (to micromolar concentrations) and by comparing the methods to determine which method is more sensitive. This can be additional evidence to bolster the authors' claim.

REQUESTED REVISIONS:

As I mentioned earlier, finding out the lower as well as the upper limit of quantification with this method would be important. This can be done by serially diluting a known concentration of Mtx. The authors can also calculate the intraassay variation by replicating the diluent and interassay variation by running this detection method on two different time points. Since by serial dilution you can predict the concentration in the solution this will bolster the authors' claim and also provide evidence that the upper limit of quantification is unaffected.

ADDITIONAL REQUESTS/SUGGESTIONS:

Some minor typos can be corrected. The discussion can be expanded.
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.
No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.
No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.
Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.
Not relevant to this manuscript

Quality of written English
Please indicate the quality of language in the manuscript:
Needs some language corrections before being published

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?
If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

This reviewer has been recruited by a partner organization, Research Square. Reviewers with declared or apparent competing interests are not utilized for these reviews. This reviewer has agreed to publication of their comments online under a Creative Commons Attribution License attributed to Research Square and was paid a small honorarium for completing the review within a specified timeframe. Honoraria for reviews such as this are paid regardless of the reviewer recommendation.

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal