Reviewer’s report

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

Version: 1 Date: 29 Oct 2018

Reviewer: Zhigang Zhao

Reviewer's report:

The manuscript (PHAT-D-18-00065R1) has been revised according to my suggestions. However, I still have 3 suggestions as follows:

1. The 1.00, 0.80, 0.67, 0.50, 0.33, 0.20, 0.10 μmol/L should be described as quality control samples for clarity.

2. In clinical practice, MTX plasma concentration is usually higher than 1 μmol/L 24 h after infusion. The matrix was different between the original and modified methods, therefore, the results for dilution in the commercial kit might not represent the influence of dilution for modified method.

3. We could ignore the relationship between difference and magnitude, the analysis will still give limits of agreement, but they will be wider apart than necessary for small plasma concentrations, and rather narrower than they should be for higher plasma concentrations.(1) Therefore, logarithmic transformation should be performed to get an appropriate 95% limits of agreement.

Reference

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

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.

I am able to assess the statistics

Quality of written English
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