Reviewer's report

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

Version: 0 Date: 17 May 2018

Reviewer: Zhigang Zhao

Reviewer's report:

Comments: The author described a sensitive EMIT assay for MTX analysis via reduction of kit reagents, and compared the agreement between modified EMIT assay and the parent assay or HPLC-MS/MS method. The language should be modified by a native speaker. The comments are listed below:

Major:

1. In "4. Programming the Viva-E Instrument", the author described that "As a result, the new calibrator set of modified EMIT assay were changed to 0, 0.05, 0.10, 0.25, 0.50, 1.00μmol/L within a scope of 0.05 to 1.00μmol/L", however, the linearity was evaluated at 1.00, 0.80, 0.67, 0.50, 0.33, 0.20, 0.10 and 0.05μmol/L. I was confused, which calibrator the author was used for MTX monitoring?

2. In "4. Programming the Viva-E Instrument", the author said that "the samples with concentration more than 1.00μmol/L should be diluted into the scope of calibration curve (0.05-1.00μmol/L) with a dilution factor 10 or 100", however, no support results were supplied.

3. In Table 3, The author said that the Hemolysis did not affect the accuracy of assay. In fact, MTX is significantly distributed in erythrocytes, therefore, hemolysis will dramatically affect the results. I think the conflict results were mainly due to the difference between the preparation of hemolytic samples used in this study and the real hemolytic sample in clinical practice. In fact, what the author evaluated is the effect of hemoglobin on MTX assay. I suggest to remove the content of Hemolysis to avoid misleading of our readers.

4. The author used the Bland-Altman plot for method comparison, however, the results were missing. From Figure 2b, we could see that the difference was increased with the increase of magnitude. Therefore, both measurements should be logarithmic transformed before analysis to get an appropriate lower limit of agreement.[1, 2] Moreover, the sample size for Bland-Altman plot is missing in Figure 2b.
5. The main metabolites of MTX is 7-OH MTX, which has a very similar chemical structure to its parent drug. Moreover, the plasma concentration of 7-OH MTX is comparable with MTX 24 hours after infusion, especially in patients with high-dose MTX therapy. Therefore, the assay should evaluate the influence of 7-OH MTX on MTX measurement.

6. Discussion of the method comparisons could have been more in-depth.

7. The English grammar should be corrected by a native speaker.

Minor:

Abstract:

1. Line 11: "Methotrexate(MTX)" should be corrected as "Methotrexate (MTX)". Please check throughout the manuscript.

2. Line 13: "The blood concentration of MTX are used" should be corrected as "The blood concentration of MTX is used".

3. Line 15: "the regime" should be revised as "the regimen".

4. Line 17: A space is usually added between numbers and units, 0.30µmol/L should be corrected as 0.30 µmol/L. Please check throughout the manuscript.

5. Line 28: "in low concentration range" should be revised as "in low concentration".

6. Line 37: "detected" should be revised as "via detection of".

7. Line 46: "Intraassay and interassay imprecision show high accuracy", in fact, imprecision could not show the accuracy of the method.

8. Line 52: "R2=0.9994" should be revised as "R2=0.9994".
Background:

1. Line 31: "time intervals" is not suitable, "time points" might be better.

2. Line 36: A space is usually added between numbers and units, 0.10µmol/L should be corrected as 0.10 µmol/L. Please check throughout the manuscript.

3. Line 47: Secondly, the cost of MTX assay on Viva-E is too high to afford routine tests for hospital's laboratory. In fact, the price for MTX assay is 95 yuan in China, it is not too high for most of our patients to afford. Moreover, about 85% of the cost is covered by the medical insurance.

Methods:

1. Line 16: "Three Quality control (QC) was" should be revised as "Three Quality controls (QC) were".

2. Line 18: "an additional custom QC 0.05μmol/L" should be revised as "an additional custom QC at 0.05μmol/L was obtained".

3. Line 36: "not any", revised as "no".

4. Line 38: "while", revised as "and".

5. Line 40: add "to" after "according".

4. Programming the Viva-E Instrument, Line 25: "many theses have reported this method" should be revised.

6. Line 56: "scope" revised as "range".

7. Line 3: "a dilution factor 10 or 100" revised as "dilution factors of 10 or 100".

5. Intraassay and Interassay Imprecision:
6. Intraassay and Interassay Imprecision: Intra-day and inter-day accuracy and imprecision might be more suitable for the content. Please check throughout the manuscript.

8. Line 22: "three samples" should be revised.

7. The LC-MS/MS Assay Procedure

9. Line 1: The following sentence should be revised: "The ion source was in positive ion mode and MRM scan was applied."

10. The Tables and Figures should be self-explanatory. Please check.

11. In Table 3, "Lipemic" should be revised as "Bilirubin", and "Icteric" should be revised as "Triglyceride" for consistency. Please check throughout the manuscript.

References:


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
Please indicate the quality of language in the manuscript:

Not suitable for publication unless extensively edited

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