Reviewer's report

Title: Evaluation of Entamoeba histolytica Recombinant Phosphoglucomutase Protein for Serodiagnosis of Amoebic Liver Abscess

Version: 1 Date: 12 February 2013

Reviewer: Tengku Shahrul Anuar

Reviewer's report:

MAJOR COMPULSORY REVISIONS

Statistical Analysis

1) The authors should analyse using Kappa statistic (K) in terms of Chi-square/Fisher's exact test. Result from the Kappa statistics will give a clear indication to the readers whether the current technique (proposed by the authors) is in agreement with the reference technique (IHA) used. The weighted Kappa statistic was used to assess the agreement between the tests; values of <0.20 indicate poor agreement between methods, 0.21-0.40 fair agreement, 0.41-0.60 moderate agreement, 0.61-0.80 good agreement and 0.81-1.00 very good agreement (Altman, 1991). A p-value <0.05 were considered as the level of significance.

2) Table 1. Sensitivity and specificity of each potential antigenic protein bands of CSA.

Reviewer: The authors calculated the sensitivity for ~170kDa (75%) and ~70kDa (75%) WRONGLY.

Formula for sensitivity: True positive / (True positive) + (False negative) x 100

3) Table 3. Comparison between rPGM-ELISA and IHA in terms of sensitivity and specificity in diagnosis of ALA using statistical analysis.

Reviewer: The authors should tabulated the results for Kappa statistic (K). Apart from that, the authors should also report the positive and negative predictive values (PPV and NPV).

MINOR ESSENTIAL REVISIONS

i. Abstract

A. Background

1) 3rd sentence: ...circulating amoebic antigens or antibodies; however those tests show...

Reviewer: Authors should change it to ...circulating amoebic antigens or antibodies. However, those tests show...
2) 4th sentence: ...was reported to be useful in diagnosis of ALA, however high antibody...
Reviewer: Authors should change it to ...was reported to be useful in diagnosis of ALA. However, high antibody...

B. Results

1) 2nd sentence: Recombinant PGM showed 79.17% (19/24) sensitivity...
Reviewer: Authors should change it to Recombinant PGM (rPGM) showed 79.17% (19/24) sensitivity...

Question: Which terminology is correct, rPGM or rPGM-ELISA?

Question: Authors should specify/state which COV? Is it based on mean +1SD or +2SD?

ii. Background

1) 2nd paragraph: Authors should standardize the use of initial e.g or i.e? Please also look at page 12 (Result-IgG blots of CSA), page 14 (Discussion) and page 15 (Discussion).

2) 4th paragraph: The corresponding gene was then cloned and expressed, followed by the diagnostic potential evaluation of the purified recombinant protein in an indirect ELISA format.
Reviewer: Please rephrase this sentence.

iii. Material and Methods

Human Serum

1) 1st sentence: detectable abscess by ultrasound imaging; and positive serology...
Reviewer: Please delete the sign ;

Question: Why the authors used IHA instead of other antibody kits such as RIDASCREEN etc? Any specific reasons?

2) 4th sentence: In addition, a total of 197 blood donor serum samples were obtained from Department of Haematology and Blood Transfusion Unit at HUSM.
Question: Is there any written informed consent? Authors need to clarify this in a sentence.

Maintenance of E. histolytica and preparation of crude soluble antigen (CSA)

1) HM-1:IMSS should not be italic

Analysis of CSA antigen protein profile via SDS-PAGE nad Western blotting

1) 3rd sentence: Subsequently, the NC membrane was out into multiple strips and incubated with human sera at a dilution of 1:200 in TBST for 2 h at RT.
Question: What is TBST? Is it 0.1% Tween 20? Authors should define it.

2) 8th sentence: The sensitivity and specificity of the antigens protein was then evaluated based on serum samples collected from ALA cases (n=24), patients with infections other than amoebiasis (n=28) and blood donors (n=30).
Question: Why blood donor only (n=30)? The authors should justify it.

Development and evaluation of indirect recombinant antigen based ELISA

1) 11th sentence: The sensitivity and specificity of the recombinant antigen in an indirect ELISA format was then evaluated against the IHA kit using the different categories of serum samples collected.
Reviewer: Authors need to rephrase this sentence.
Question: Is IHA the reference technique used in this study? Authors need to make a clear statement in the sentence.

iv. Results
IgG blots of CSA
1) 2nd sentence: ...when tested against a total of 63 negative control serum samples.
Question: Why only 63 and not 197) Authors should justify it.

2-DE western blot analysis and protein identification
1) 2nd sentence: Since the ~170 kDa was most probably...
Question: How about ~100 kDa? Authors need to make a clear discussion about it.

Efficacy of rPGM-ELISA
1) 5th sentence: In comparison with IHA kit, statistical analysis showed that...
Reviewer: Authors need to refer to the MAJOR COMPULSORY REVISIONS

v. Discussion
1) 4th paragraph: Authors need to change 'false positivity' to 'false positive'
Reviewer: Authors should discuss briefly about ~100 kDa at the end of the paragraph.

2) 7th paragraph: According to Flores et al. (1993)
Reviewer: Authors need to check the reference no.

3) 7th paragraph (sentence no.8): The validity is almost similar with rPGM protein in the current study, which is 79.2% sensitive and 86.7% specific.
Reviewer: Authors should standardize either to use 79.17% or 79.2% and 86.7% or 86.67%. 
4) 7th paragraph (sentence no.14 and 15): A recombinant 29 kDa cysteine-rich surface protein was also developed...
Question: Any specific references?

5) 8th paragraph (sentence no.2): In the current study, the novel rPGM protein showed diagnostic validity of ~80% sensitivity and ~90% specificity in diagnosis of ALA.
Reviewer: Authors need to change it to 79.2% or 79.17% and 86.7% or 86.67% (Please refer to comment no. 3 above).

vi. Acknowledgements

1) 2nd sentence: The first two authors (PLEASE USE INITIAL) received financial support through the university fellowship and the fifth author (PLEASE USE INITIAL) is a graduate assistant at School of Health Sciences.

vii. References

1) References in the text: [15, 16]
Reviewer: Authors should followed the BMC Infectious Diseases journal style e.g. [15,16]. Please check for the whole manuscript.

2) References (Page 21):
i. Please check references no 2, 3, 12 and 32. Please use initial/short form for the journal title and follow the BMC Infectious Diseases journal style.

ii. Please italic the GENUS and SPECIES (Entamoeba histolytica) in the reference no 5, 7, 9, 10, 14, 15, 16, 17, 22, 23, 24, 25, 27, 29, 30, 31, 33, 34, 35, 36, 38, 39.

viii. Additional comments

1) Authors should follow the BMC Infectious Diseases journal writing format e.g. second paragraph onwards need to click TAB. Please check for the whole manuscript especially in Background and Discussion section.

2) Discussion is too long. Please delete and rephrase it when appropriate.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests