Reviewer’s report

Title: Evaluation of Fluorescent In-Situ Hybridization Technique for Diagnosis of Malaria in Ahero Sub-County Hospital, Kenya 2016

Version: 0 Date: 07 Aug 2017

Reviewer: Lemu Golassa

Reviewer's report:

I have reviewed the manuscript by Kandie et al., "Evaluation of Fluorescent In-Situ Hybridization Technique for Diagnosis of Malaria in Ahero Sub-County Hospital, Kenya, 2016. In this manuscript authors evaluated the diagnostic performances of In-Situ Hybridization (FISH) against microscopy, RDT and PCR. The advantages and disadvantages of each of the malaria diagnostic tests (microscopy, RDT and PCR) have been reviewed. This necessitate the search for complement malaria diagnostic tools like FISH. The title is interesting and the connotation of adoption of FISH technique for malaria diagnosis to complement the existing malaria diagnostic methods is worth mentioning.

Blood samples were collected from febrile patients attending Ahero Sub-County Hospital in Kenya and interrogated using (FISH), microscopy, RDT and PCR. Taking microscopy and/or PCR as the gold standard, authors reported 85.6% sensitivity and 96.2% specificity of FISH.

Points to address

1. The formula used to calculate the sample size does not seem correct (n≥ d2 *p (1-p)/d2) and it should read n= d2 *p (1-p)/d2. When was this study conducted?

2. It is not clear whether authors used Plasmodium genus FISH (P-Genus FISH) kit, or P. falciparum FISH kit, or P. vivax FISH kits or whether each sample was tested by P-Genus or PF or PV FISH assays? If all these assays were carried out, details of the procedures followed and analytical sensitivity of the assays are required.

3. The prevalence of malaria was reported, however, the species composition of the different Plasmodium was not mentioned in the report.

4. Ninety or 29.8% (in line 21 of result section) of the samples were positive for malaria parasites by microscopy and/or PCR; this needs paraphrasing.

5. Table 1 reports the percentages of malaria positive and negative patients by different tests; however reporting the percentage of negative patients does not seem standard way of presentation.
6. Table 2a & b. The diagnostic performances of FISH, microscopy, RDT, and PCR could have been presented against the assumed gold standard test(s) (i.e. microscopy &/or PCR) in a 2 x 2 table. Furthermore, the captions of all the tables do not seem conveying the necessary information they are supposed to do (refer to Table 1, 2a, b, etc). Indeed, captions should able to convey the necessary information.

7. Authors reported the presence of high level of agreement between FIFH and the rest of the methods. In Table 4, the level of agreement among the diagnostic tests do not seem correct. According to Table 4, for instance, of 85 FISH positives, it is calculated from this table that Giemsa-Microscopy detected 17 of them as positives, Giemsa-Microscopy & PCR 8 of them; RDT 11 of them, etc. and this does not support the presence of agreements between tests. To portray agreements between FISH versus Giemsa, PCR & RDT, authors may use table below to depict it.

8. What was the performances of FISH by age group and level of parasitaemia? Additional info of patients (patients age group, sex) would insight the diagnostic performance of the tests.

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

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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

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