Peer Review File

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Reviewer A

Comment 1: Using the sensitivities and specificities from the reviewed literature featured in Tables 1-5, include this information in the text in a way that supports the argument. Why are the current diagnostic practices in the Philippines are insufficient for detecting early infections/low parasitemia? Elaborate the advantages and disadvantages of multiple methods from other papers. What are the methods that are preferable and would successfully fill existing gaps in malaria detection due to low parasitemia?

Reply 1: In the results, we added some discussions (**3.7 Sensitivity and specificity of conventional diagnostic tests**) about the sensitivity, specificity, advantages, and disadvantages of diagnostic tests with conventional targets (e.g., *Pf*HRP2, pLDH, Hemozoin) with microscopy or PCR as the gold standard (see **Page 12-13**, **lines 214-237**). We also added a discussion **(3.8 Sensitivity and specificity of DNA/ssRNA based tests)** about the sensitivity and specificity of DNA/ssRNA based tests with microscopy or PCR as the gold standard (see Page **13-14**, **lines 238-252**).

Changes (additions) in the text 1:

3.7 Sensitivity and specificity of conventional diagnostic tests

Regarding the summary of studies on the performance of RDTs (with microscopy as the gold standard) in symptomatic patients, it was stated that some commercial RDTs that detect *Pf*HRP2 (e.g., ParaSight®, SD Bioline, etc.) have a sensitivity ranging from 81% to 94% and a specificity from 41% to 100% (23–28); see *Table 2*. In addition, some RDTs that detect pLDH (e.g., Optimal) have a sensitivity ranging from 39% to 97% and a specificity from 59% to 97% (33,34); see *Table 3*. Lastly, some commercial devices that detect hemozoin (e.g., Cell-Dyn and Gazelle™) have a sensitivity ranging from 72% to 96% and a specificity from 83% to 100% (38,41); see *Table 4*. However, the performance of these methods was not determined in a study that included asymptomatic participants who lived in malaria endemic areas. In contrast, field surveys using RDTs among asymptomatic participants in malaria endemic areas produce more varied results. RDTs that detect *Pf*HRP2 (e.g., ParaCheck Pf and ParaHIT f) had a sensitivity ranging from 5% to 67% and a specificity from 83% to 100%, while RDTs that detect pLDH (e.g., Optimal) had a sensitivity ranging from 11% to 97% and a specificity from 18% to 92% (35). With all these variable performances in the field, malaria case identification, their management, and overall efforts on control and elimination can get compromised. Possible factors that caused this variability are adverse environmental conditions (e.g., humidity, temperature, etc.) that led to the deterioration of equipment (e.g., test strips), or random errors caused by the personnel performing the procedures (35). Regarding the performance of RDTs that detect *Pf*HRP2 (with a real-time PCR or qPCR) as the gold standard in asymptomatic participants, it had a sensitivity of 76% and a specificity of 97% (29); see *Table 2*. A similar study, with a conventional (qualitative) PCR as the gold standard had a sensitivity of 98% and a specificity of 99% (30). Nonetheless, the RDTs used in these studies can potentially produce false negative results from *Pf*HRP2 gene deletion leading to undiagnosed infections with *P. falciparum* (71,79). Therefore, a more advanced commercial method that can detect malaria DNA/ssRNA (e.g., PCR, LAMP) in blood samples regardless of mutations could improve the overall diagnostic performance.

3.8. Sensitivity and specificity of DNA/ssRNA based tests

Regarding the summary of studies on the performance of DNA/ssRNA based tests (with microscopy as the gold standard) on symptomatic patients, one study stated that the sensitivity and specificity of commercial qPCR methods (e.g., QuantiFast™ and abTES™) had an average of 99% (51). In another study, commercial LAMP methods (e.g., Illumigene® and Loopamp™) with a conventional PCR as the gold standard had a sensitivity and specificity averaging at 97% and 91%, respectively (55,56). However, this studies however did not include asymptomatic participants. Nonetheless, in the study of Reyes et al., PCR has detected more

malaria infections (36.7% and 38% higher) than microscopy and RDTs, respectively (4). In addition to their greater sensitivity, PCR-based tests have the potential to determine parasite load, species, and drug resistance. LAMP in contrast, though it requires less equipment, it cannot determine for parasite load and mutations since its only qualitative (70). Overall, these NAATs may have the higher detection rates of asymptomatic malaria with residual parasitemia, and that they could be more useful in countries gearing towards malaria elimination. However, they are also subject to variability in sample preparation, the amplification step, and the read-out of the results; thus, their variants (e.g., real-time, multiplex, nested, etc.) may also vary in sensitivity and specificity (70).

Comment 2: I encourage the authors to also consider in more detail the feasibility of introducing a new technology, as briefly mentioned here. What is the capacity to implement this technology in the Philippines on a national and/or regional scale in terms of cost, laboratory space, and workforce?

Reply 2: There was no published local research about the feasibility of introducing a new technology as well as the capacity in terms of cost, laboratory space and workforce in the Philippines. But we added some discussions (**3.9 Financing Malaria Control**) about the financial support which may affect to the implementation of this technology (NAATs) in the Philippines. (see **Page 14**, **lines 253-266**)"

Changes (additions) in the text 2:

3.9 Financing Malaria Control

Regarding the trends in the Philippines domestic financing and international disbursement in relation to meeting global malaria control targets, funding has been decreasing in the past decade. The Global Fund is the single largest source of funding for malaria, accounting for 60.4% of total disbursed funding from 2010 to 2019. The funds for malaria control from the Global Fund and other non-government contributions decreased from US$ 22.6 million in 2010 to US$ 3.4 million in 2019. Available information suggests that domestic funding is generally less than US$0.5, with an average of US$0.23 per person at risk from 2010 to 2019. (1,6–15). Regarding the financing of malaria diagnostic tests, though cost is an important factor when suggesting a preferable methods that may fill existing gaps in asymptomatic malaria detection, this factor was not included in the review as additional data would be required to estimate the costs per assay, including maintenance of facilities, equipment, and reagents in the Philippines. Lower numbers of reported malaria cases may have led to the reductions in funding. Therefore, the implementation of newer technology in the national or regional scale may be possibly hindered by an inadequate financial support.

Comment 3: In section 3.5 or the discussion, it would be pertinent to mention pfhrp2 deletion phenomena which can lead to false negative RDT results (parasites were present but HRP2 was not expressed due to the gene not being present).

Reply 3: We added some Statements. See **Page 10 (line 172-174)** and **page 13 (line 234-235)**

Changes (additions) in the text 3:

Page 10 (line 172-174). However, one of the emerging concerns for malaria diagnosis are the worldwide reports of *Pf*HRP2 gene deletion that can lead to false negative results. This may affect malaria control and elimination efforts in the Philippines (71).

Page 13 (line 234-235). Nonetheless, the RDTs used in these studies can potentially produce false negative results from *Pf*HRP2 gene deletion leading to undiagnosed infections with *P. falciparum* (71,79).

Comment 4: Lns 218-219, **221-222**, **234-235**: There appear to be contradictions in the text as to whether the Philippines confirms every suspect malaria case.

Reply 4: We have **deleted** the text "If the parasitological diagnosis is not accessible, antimalarial treatment can be done according to clinical suspicion, though treatment is usually done following diagnostic testing" in the former lines **221-222** of **3.7** Status of Malaria Diagnosis in the Philippines. But we have not modified the text in the former lines **234-235**. In 234-235, there was an abrupt drop only in "Reported" RDT use in 2019 and 2020, possibly due to incomplete or partial data reported in that period by the NMCEP to the WHO

Changes in the text 4:
The test is no longer seen in the lines 271 of the current **3.9** Status of Malaria Diagnosis in the Philippines

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Comment 5: Ln 236: Please explain the annual blood examination rate (ABER) in the text. Citations must be provided for these statistics.

Reply 5: We have modified our text as advised (see Page 15, line 285-287)". We also inserted the citations from the sources of the data.
Changes (additions) in the text 5:
Lastly, the ABER (Annual blood examination rate) which refers to the number of parasitological tests (by microscopy or RDT) undertaken per 100 people at risk per year (expressed as percentage) was on average of 0.6% only (1,6-15).

Comment 6: Lns 248-254 (4.1 Key finding): Please include key findings extrapolated from the systematic review.

Reply 6: We have modified our text as advised (see Page 16, line 304-306)

Changes (additions) in the text 6: Nonetheless, the RDTs used by the NMCEP for identifying malaria cases may potentially produce false negative results due to emerging mutations of target biomarkers (e.g., PfHRP-2) leading to undiagnosed P. falciparum infections.

Comment 7: Line 25 (in the abstract) remove the citation number and change to "PRISMA 2020 guidelines"

Reply 7: We have modified our text as advised (see Page 2, line 25)

Changes in the text 7: We have modified our text from "PRISMA guidelines 17" to "PRISMA 2020 guidelines"

Comment 8: qPCR is mentioned only in the conclusion (former line 327), whereas conventional PCR is focused on in section 3.6 – please adjust to PCR in the conclusion.

Reply 8: We have modified our text as advised (see Page 20, line 381)"

Changes in the text 8: We have modified our text from "**qPCR**" to "**PCR**"

Comment 9: Reference 16 (WHO World Malaria Report, 2020) is a duplicate of reference 1.

Reply 9: We removed the duplicate reference.
Changes in the text 9: "Reference 16 (WHO World Malaria Report, 2020)" was removed and we have adjusted the referencing number.

Reviewer B

Comment 1. "…by Jaureguiberry et al. in 1990, and in the form of LAMP by Poon, Wong, and Ma in 2006 (53)."

*Jaureguiberry et al. should have a citation.

*The author name and published year of Ref 53 should be double-checked and make sure they are aligned with the bibliography.

Reply 1. We have modified our text as advised, (see **Page 12, lines 209-210**). We also have checked their alignment with the bibliography.

Changes in Text 1: Eventually, NAATs for malaria diagnosis were developed, such as in the form of a conventional PCR by Jaureguiberry et al. in 1990 (44), and in the form of LAMP by Poon, Wong, and Ma in 2005 (52).

Comment 2. "…using the data derived from the World Malaria Report 2009 to 2020 (1,6-15)."

There is no WHO report of 2009 in the bibliography. Please confirm whether it should be replaced with 2010.

Reply 2. We have modified the text and updated the citation (see **Page 6, lines 75-75**). Note: The World Malaria Report 2021 contains the updated data for the year 2020.

Changes in Text 2: …using the data derived from the World Malaria Report 2010 to 2021 (1,2,6-15).

Comment 3. "Studies related to malaria biomarkers or targets and the development of its detection methods until November 2022 were systematically searched in MEDLINE through PubMed."

December was reported in the Abstract instead of November. Please confirm which one is correct.

Reply 3: We have corrected our text as suggested (see **Page 6, lines 80-81)**

Changes in Text 3: Studies related to malaria biomarkers or targets and the development of its detection methods until **December 2022** were systematically searched in MEDLINE through PubMed

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Comment 4. "P. falciparum levels in whole blood hemolysates and plasma of malaria patients was published in the year 1993 by Makler and Hinricks."

A citation is required for the above sentence.

Reply 4. We have cited the sentence as suggested (see Page **10**, **lines 183-184**).

Changes in Text 4: "……*P. falciparum* levels in whole blood hemolysates and plasma of malaria patients was published in the year 1993 by Makler and Hinricks (31)"

Comment 5. Please check the correctness of the author names and published years of the below contents.

“Finally, the detection of hemozoin (malaria pigment) was first reported in 1983 by Jamjoom et. al. Hemozoin is a heme crystal produced by Plasmodium spp. when it digests hemoglobin (36).”

Reply 5: We have modified the text. see Page **11**, **lines 186-187**). There was only 1 author (no co-author). We had confirmed the correctness of the published year.

Changes in Text 5: Finally, the detection of hemozoin (malaria pigment) was first reported in 1983 by G. Jamjoom. Hemozoin is a heme crystal produced by *Plasmodium spp*. when it digests hemoglobin (36).

Comment 6. “…, it was in the year 1984 by Franzén et. al., by using probe hybridization and dot blot hybridization assays (42).”

Reply 6: We have confirmed the correctness of the author names and published year. We also have modified the text (see Page **12**, **lines 208-209**).

Changes in Text 6. …,it was published in 1984 by Franzén et al., using probe hybridization and dot blot hybridization assays (42)

Comment 7. “…1991 by Parra et. al.”

Reply 7: We have confirmed the correctness of the author names and published year (see **Page 12**, **lines 177 -178**).

No Changes in Text 7.

Comment 8. “Available information suggests that domestic funding is generally less than US$0.5, with an average of US$0.23 per person at risk from 2010 to 2019. (1,6–15).”

Please check whether 2019 should be replaced with 2020.

Reply 8: We have modified our text as advised. We also updated the citation (see **Page 14, line 266-268**)
Changes in Text 8: Available information suggests that domestic funding is generally less than US$0.5, with an average of US$0.23 per person at risk from 2010 to 2020. (1,2,6–15).

Comment 9. “[with a real-time PCR (qPCR)]” - the definition and abbreviation are not matched.

Reply 9: We have modified our text as advised (see **Page 13, line 239**)

Changes in Text 9: (with a real-time PCR)

Comment 10. “Among all the included studies, only 2 studies **(one study each for hemozoin and LAMP)** have used *Pf*HRP-2 RDT as the gold standard (usually along with microscopy and/or PCR”**.** Please review the sentence (in BOLD).

Reply 10: For clarity of statement, we **removed** the **words in bold letters** and added citations instead (see **Page 10, line 175-176**).

Changes in Text 10: Among all the included studies, only two studies have used *Pf*HRP-2 RDT as the gold standard, usually along with microscopy and/or PCR (39,56).

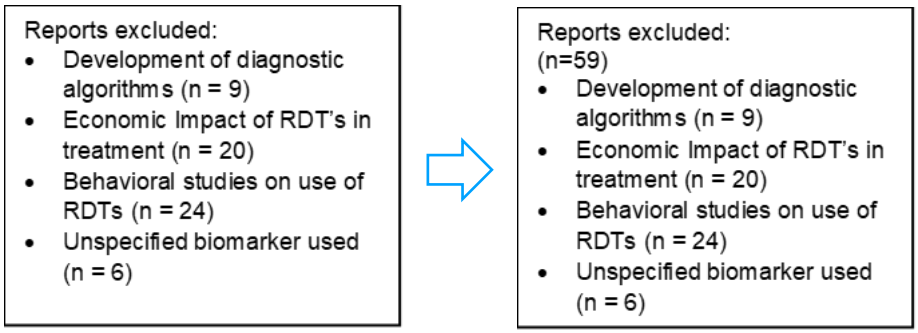
Comment 11: "However, **this studies** however did not include asymptomatic participants." Please confirm whether singular or plural should be used.

Reply 11: We have modified the text as advised (see **page 14, lines 251-252**).

Changes in Text 11: However, **these** studies did not include asymptomatic participants.

Comment 12. "Following the screening, additional **59 articles** were excluded from the remaining 100 articles with reasons such as the studies were about the development of diagnostic algorithms"
59 does not correspond with the data reported in Figure 1.

Reply 12: We have modified Figure 1, to highlight the number of excluded articles [see the File**, Palmares, Aaron Jan (Figures).docx**].



Changes in the Figure 12: We indicated the **Reports excluded: n=59** in the figure

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Comment 13: RDT/RBC/LAMP/QBC/PCR/pLDH/ELISA/NMCEP/LINN should be defined upon first use in the Main Text.

Reply 13: We have the defined the abbreviations upon its first use in the Main Text as suggested

Changes in Text 13:

- rapid diagnostic tests (RDTs) - see page 5, line 62
- red blood cells (RBC) - see page 7, line 115
- loop-mediated isothermal amplification (LAMP) - see page 7, line 102
- quantitative buffy coat (QBC) - see page 8, line 116
- polymerase chain reaction (PCR) - see page 7, line 102
- parasite lactate dehydrogenase (pLDH) - see page 5, line 68
- *Plasmodium falciparum* histidine-rich protein (*Pf*HRP2) - see page 5, line 67-68
- enzyme-linked immunosorbent assay (ELISA) - see page 11, line 196
- National Malaria Control and Elimination Program (NMCEP) - see page 16, line 311-312
- long-lasting insecticidal nets (LLIN) - see page 18, line 353

Comment 14. All the abbreviations in the figure(s) and table(s) should be defined in the explanatory legend.

Reply 14: We have the defined the abbreviations for all Figures and Tables in the explanatory legend. See Files of Figures and Tables "**Palmares, Aaron Jan (Figures).docx**" and "**Palmares, Aaron Jan (Tables).docx**"

Comment 15. The final number of the included studies is not correct. Please check and revise.

Reply 15: We have confirmed the correctness of the number of included studies (see **Page 7-8**, **lines 114-121**).

5 studies - malaria-infected RBCs (**16**–20)
10 studies - *Pf*HRP-2 only (21–30)
4 studies - pLDH only (31–34)
1 study - both PfHRP-2 and pLDH (35)
6 studies - Hemozoin (36–41)
15 studies - DNA/ssRNA genes (42–**56**).
41 studies in total

Citations were numbered from **16** to **56**. There was 1 study "35. Belizario et al., 2005" that was included in both Table 2 (*Pf*HRP2) and Table 3 (pLDH) since they studied the performance of both markers. See Table 2 and Table 3 in the File "**Palmares, Aaron Jan (Tables).docx**"