



SDI Review Form 1.6

Journal Name:	International Journal of TROPICAL DISEASE & Health
Manuscript Number:	Ms_IJTDH_18744
Title of the Manuscript:	Detection and species identification of malaria parasites by nested-PCR: comparison with light microscopy and with SD BIOLINE Malaria Ag test in Luanda, Angola.
Type of the Article	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	While this is an interesting article and potentially useful, the authors should comment about cost comparisons between the different assays (I assume that the nested PCR is much more expensive than the other two); also the time involved from obtaining samples to getting results should be discussed. This could be a very important issue since test results impact the drugs/therapy given to the patients.	Thank you very much for your comments; with these the paper leaves great quality. We hope answer all comments and insert in the manuscript from rich that. We added some comments about the time, cost and labour consuming in the conclusion section. " In this study we demonstrated that RDT were significantly more sensitive than microscopy, this test is a rapid diagnostic test, are not labor-intense but are more costly than microscopy. These findings support the need of using RDT in the diagnosis of Plasmodium. However the sensitivity of microscopy was higher in comparison with other studied reported in Angola may be for the microscopist training course developed before the study. The nested-PCR is time- and labour-consuming and required expensive reagents and infrastructure. However, our results confirmed the considerable sensitivity of nested-PCR for detection of cases that remain undiagnosed by microscopy and RDT. PCR could appear to be a useful method for detecting Plasmodium parasites during active malaria surveillance in Angola. This study



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	More information about time between blood collection (finger-prick) and analysis should be given; were the tests done on freshly obtained blood or were the samples stored? For the filter paper samples (which appear to be have sent to Cuba?), how were these samples stored?	contributes to the less literature regarding malaria species in Angola. Only single infections were detected, the <i>P. falciparum</i> infection was reported in the majority of positive cases, the other specie less detected was <i>P. malariae</i>. The finger-prick samples were obtained freshly for microscopy and SD BIOLINE Malaria Ag. For the filter paper samples the paragraph was re-write. In addition, 3-5 drops of blood were spotted onto filter paper (Whatman® 3MM Chr, Whatman International Ltd., Maidstone, England). Each filter paper sample was dried at room temperature and store at 4°C in separate plastic packets to avoid cross contamination for nested-PCR analysis. The whole blood sample and dried filter paper were collected simultaneously. The dried filter paper samples were transported to Molecular Biology Laboratory, Parasitology Department, Institute of Tropical Medicine "Pedro Kourí" (IPK), Havana, Cuba in iceboxes (0-5 °C). The DNA extraction and nested-PCR was performed at IPK.
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<u>Minor</u> REVISION comments	This manuscript has many grammatical errors, typographical errors and spelling errors. Also the material in lines 32-35 would flow better if moved to line 28 after the word “patients”. The authors should define abbreviations the first time they are used (ie Ag, PCR, bp). Lines 218-219: this is not a complete sentence and thus does not make sense.	The English was revised. The lines 32-35 were moved according to the reviewer suggestion. Was done The sentence was changed: Ten (43.5 %) of the 23 false negative microscopy samples were negative by RDT.
<u>Optional/General</u> comments	The authors should also include Dzakah et al. (<i>Malaria Journal</i> 2014, 13:272) who have recently reported that a combination of two antigens (aldolase and LDH) in RDTs for rapid diagnosis enhanced the sensitivity of the assay and reduced misdiagnosis.	The reference was included. Reference 50. In the text thi sentence was included: Recently, Dzakah et al. [50] demonstrated that a combination of aldolase and LDH in RDTs for the rapid diagnosis will enhance the sensitivity of the assay and reduce misdiagnosis. Other RDTs will be evaluated in Angola context in future researches.