# DNA Electrochemical biosensor for Plasmodium falciparum and Plasmodium vivax differential detection

Rodney Lopez Loja[1], Ceili Zuta-Chamoli[1], Katherine Lozano Untiveros[1], Stella M. Chenet[1], Juan R. Tejedo[1,2,3], Rafael Tapia-Limonchi[1]

1 Instituto de Enfermedades Tropicales, Universidad Nacional Toribio Rodríguez de Mendoza (UNTRM), Amazonas, Peru.
2 Diabetes and Associated Metabolic Diseases Networking Biomedical Research Centre (CIBERDEM), Madrid, Spain.
3 Departamento de Biología Molecular E Ingeniería Bioquímica, Universidad Pablo de Olavide (UPO), Seville, Spain.

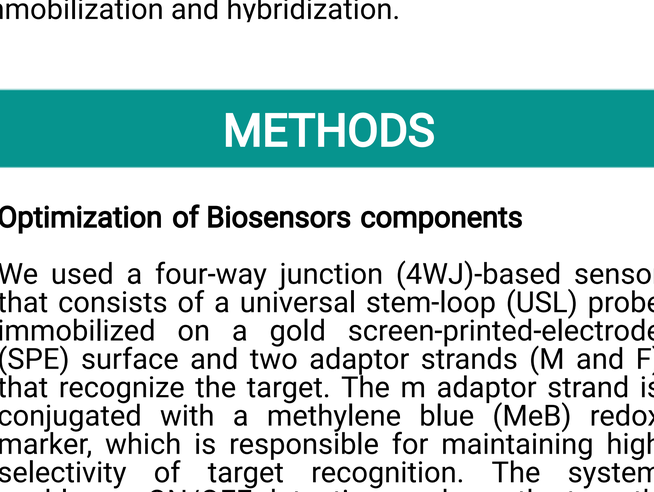
## INTRODUCTION

Current malaria rapid diagnostic tests (RDTs) for Plasmodium detection are valuable tools for point-of-care (POC) testing. However, they have some limitations that reduce their utility in low transmission settings such as, poor limit of detection (LOD) and the low sensitivity and/or specificity in areas where validation has not been performed with local samples. Thus, the development of POC devices based on electrochemical sensors provides a good alternative for the efficient detection of nucleic acids.
Here, we report for the first time the design and development of electrochemical biosensors able to detect Plasmodium and discriminate between the species P. falciparum and P. vivax by nucleic acid immobilization and hybridization.

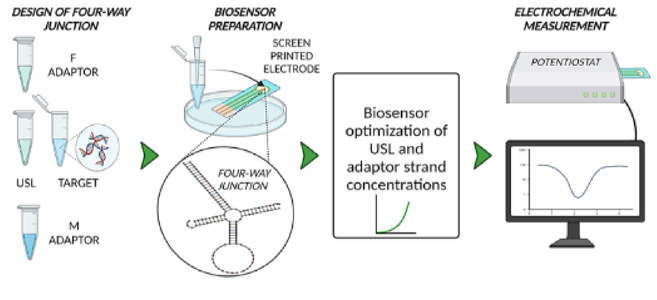
## METHODS

### Optimization of Biosensors components

We used a four-way junction (4WJ)-based sensor that consists of a universal stem-loop (USL) probe immobilized on a gold screen-printed-electrode (SPE) surface and two adaptor strands (M and F) that recognize the target. The m adaptor strand is conjugated with a methylene blue (MeB) redox marker, which is responsible for maintaining high selectivity of target recognition. The system enables an ON/OFF detection mode, as the target's presence brings the redox marker close to the SPE surface, which generates a voltage change and a current transferring by using the square wave voltammetry technique, where the signal is processed and graphed by a portable potentiostat device.

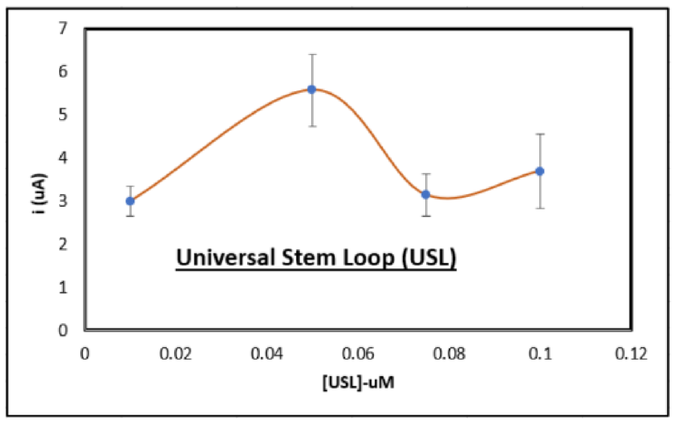



Probes were designed to target 18S ribosomal RNA sequences for Plasmodium and multi-copy gene sequences, Pvr47 and Pfr364 were considered for the detection of P. vivax and P. falciparum, respectively. Synthetic target and PCR products were used to assess biosensor performance.

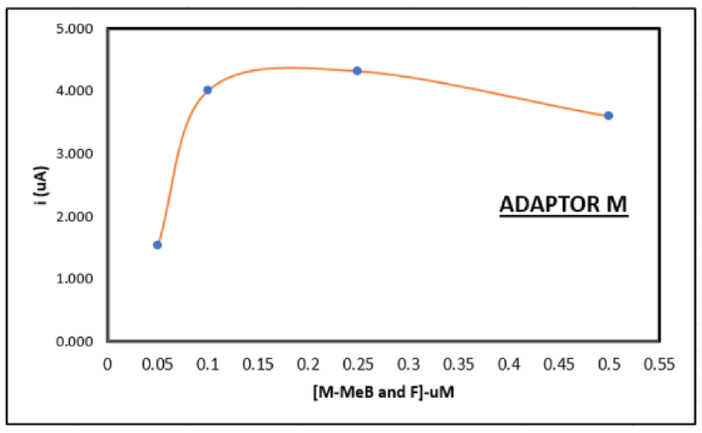


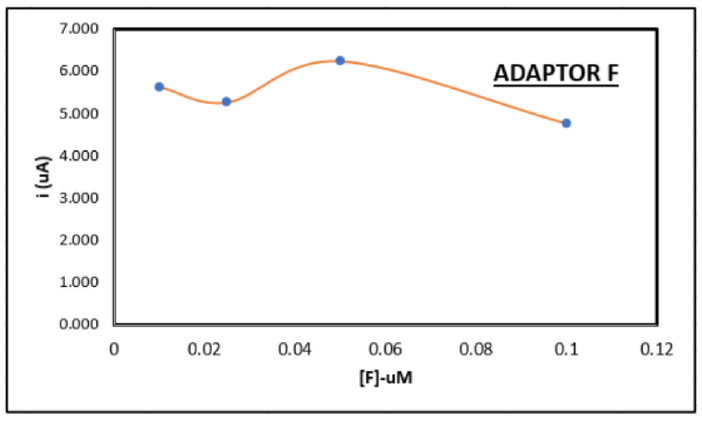

## RESULTS

For the optimization of the biosensor different concentrations of the components were standardized using calibration curves. The higher values of electric current peak (i) recorded from SWV were selected for the USL probe and adaptor strands M and F in presence of 50nM of amplified DNA target of pfr364.



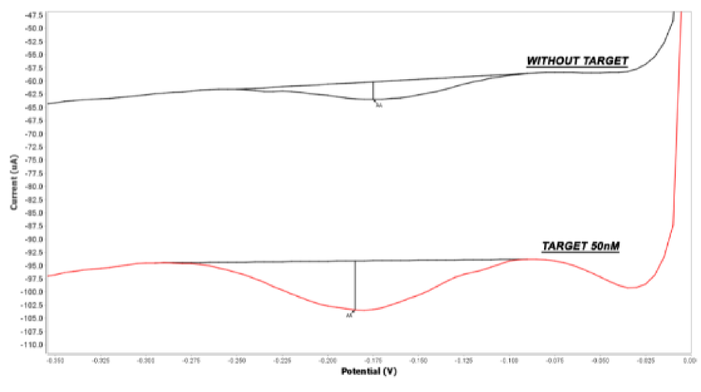

## RESULTS

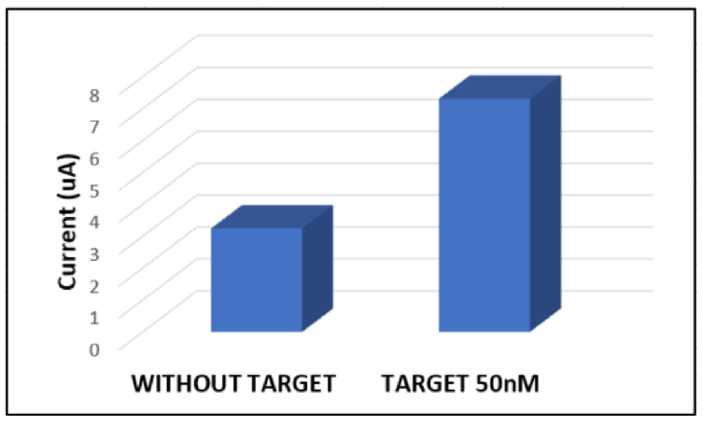



### Detection of P. falciparum and PCR amplicons

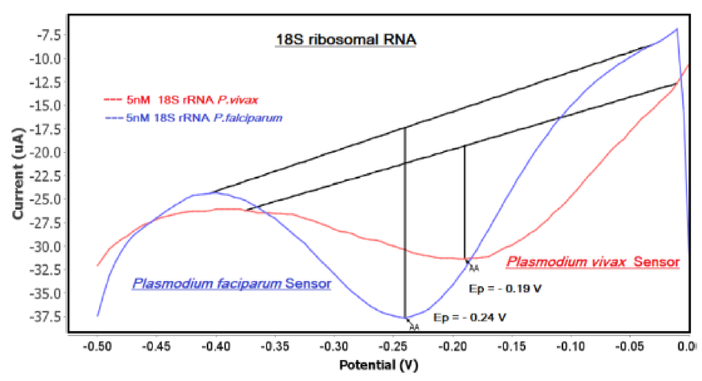
Performance of the biosensors was evaluated by SWV in a portable potentiostat. Graphics show the specificity of the sensor amplicon.



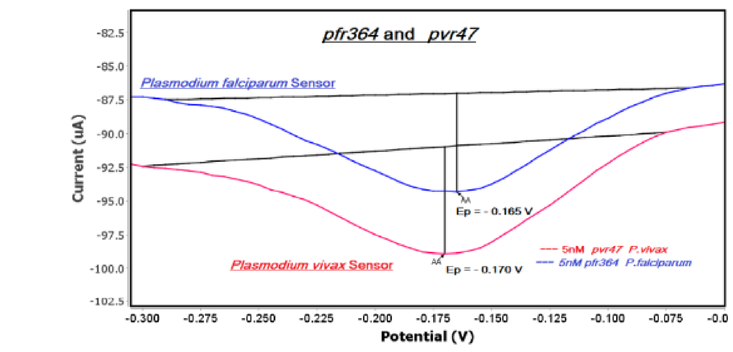




### Differential detection of P. falciparum and P. vivax

Two kind of molecular markers were evaluated: 18S ribosomal RNA, and the multi-copy gene sequences (pvr47 and pfr364) for the detection of P. vivax and P. falciparum .The redox marker methylene blue (MeB) was recorded as an ON signal when target hybridization occurred as see bellow for each biosensor. For both biosensors, the MeB potential (Ep, V) recorded was in the range of - 0.15 V to - 0.25 V.




## RESULTS




## CONCLUSION

1. A Four-Way Junction electrochemical biosensor was designed and optimized to selectively detect Plasmodium falciparum and Plasmodium vivax by nucleic acid inmovilization and hybridization.

2. The biosensors successfully interrogated the product of DNA amplification directly in the unpurified amplification from the PCR targeting pfr364, and pvr47 for Plasmodium falciparum and Plasmodium vivax respectively.

3. These biosensors enable an efficient molecular detection by recognition of specific sequences and the capability to discriminate species of a same genre, and therefore, can count with the potential to be adapted as a POC test for the molecular detection of malaria in the field.

## REFERENCES

Mills, D. M.; Foguel, M. V.; Martin, C. P.; Trieu, T. T.; Kamar, O.; Calvo-Marzal, P.; Kolpashchikov, D. M.; Chumbimuni-Torres, K. Y. (2019) Sens. Actuators, B, 293, 11–15.

Lozano Untiveros, K., da Silva, E. G., de Abreu, F. C., da Silva-Júnior, E. F., de Araújo-Junior, J. X., Mendoça de Aquino, T., Armas, S. M., de Moura, R. O., Mendonça-Junior, F., Serafim, V. L., & Chumbimuni-Torres, K. (2019).. Biosensors & bioelectronics, 133, 160–168.

Mills, D. M., Calvo-Marzal, P., Pinzon, J. M., Armas, S., Kolpashchikov, D. M., & Chumbimuni- Torres, K. Y. (2017). Electroanalysis, 29(3), 873–879.

## FUNDING

Funding by FONDECYT-BANCO MUNDIAL: CONTRATO N 009-2019-FONDECYT-BM-INC.INV

CONTACT INFORMATION

rodney.lopez@untrm.edu.pe

rafael.tapia@untrm.edu.pe