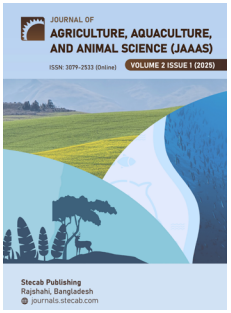

Journal of Agriculture, Aquaculture, and Animal Science (JAAAS)

ISSN: 3079-2533 (Online)

Volume 2 Issue 1, (2025)

https://doi.org/10.69739/jaaas.v2i1.468

https://journals.stecab.com/jaaas

Published by
Stecab Publishing

Research Article

Microscopic and Molecular Identification of *Plasmodium falciparum* in Patients of Wasit Province, Iraq

*[1]Thuraya Khaled Abdulwahed

About Article

Article History

Submission: March 15, 2025
Acceptance : April 21, 2025
Publication : April 27, 2025

Keywords

Malaria, Molecular, PCR, Wasit

About Author

[1] Medical Physics Department, Kut University College, Wasit-52001, Iraq

Contact @ Thuraya Khaled Abdulwahed
thuraya.khaled@alkutcollege.edu.iq

ABSTRACT

The study created electron transfer PCR, using self-quenching primers to identify several species of Plasmodium. The potential use of polymerase chain reaction (PCR) for the molecular identification of malaria parasites in Wasit, Iraq. QIAGEN extracted DNA from desiccated blood spots. PCR analysis was conducted twice on each of the 200 samples. Positive samples had a CT value of 60 or below. Fifty samples were evaluated for accuracy using nested PCR. A TaqMan-based real-time PCR technique was used on these samples. PCR successfully detected 20 positive samples from a total of 200. The samples tested positive by both nested and TaqMan methodologies. Neither PCR nor TaqMan successfully identified a positive sample in a selection of 50; however, nested PCR did. In conclusion, Nested PCR has more sensitivity than PET-PCR; nonetheless, it is not the optimal selection for high-throughput sample screening. Nested PCR serves as an effective alternative test for the quick screening of several samples in the laboratory due to its straightforward implementation and cost-effectiveness.

Citation Style:

Abdulwahed, T. K. (2025). Microscopic and Molecular Identification of Plasmodium falciparum in Patients of Wasit Province, Iraq. *Journal of Agriculture, Aquaculture, and Animal Science, 2*(1), 117-122. https://doi.org/10.69739/jaaas.v2i1.468

1. INTRODUCTION

To assess the effectiveness of intervention programs and allocate resources appropriately, the incidence and distribution of malaria infection within a nation may be used (Obaldia *et al.*, 2022). The frequency of the malaria parasite must be assessed properly (Tao *et al.*, 2022). Microscopy and several fast diagnostic procedures are used in many epidemiological surveillance studies to discover malaria parasites. In accordance with a WHO advice to postpone therapy until a parasitological diagnosis is obtained, there was a rise in the use of RDTs. Rapid diagnostic tests (RDTs) are user-friendly, do not need electricity or specialist expertise, and provide findings that inform treatment choices (Martín-Ramírez *et al.*, 2022). An asymptomatic low-density parasitic infection cannot be identified via microscopy (Gharban *et al.*, 2022).

2. LITERATURE REVIEW

Asymptomatic malaria infections may serve as transmission reservoirs in both low and high transmission environments, resulting in new infection cases (Pal-Bhowmick *et al.*, 2022). Identifying both symptomatic and silent parasite infections is essential for malaria eradication efforts (Moin-Vaziri *et al.*, 2022). Molecular methods, such as 'PCR-based assays, have superior sensitivity and specificity compared to microscopy and RDTs, enabling the detection of parasitaemia at concentrations as low as 2 parasites per liter (Pitri *et al.*, 2022). Microscopy and PCR provide lower prevalence estimates of malaria parasites compared to epidemiological investigations using molecular testing (Gharban & Ajaj, 2024). Molecular assays in extensive epidemiological surveillance investigations encounter many obstacles (Razooqi *et al.*, 2022). Molecular tests are straightforward to perform and very inexpensive. An effective molecular test may effectively screen several samples at a little cost. In contrast to conventional nested 18S rRNA PCR tests, real-time PCR techniques are more advantageous for extensive research initiatives (Gharban, 2023). The expenses associated with various test types are decreasing due to the reduction in reaction volumes and the development of more economical primers and fluorophores. The multiplexed photo-induced electron transfer PCR assay is a dependable and economical diagnostic instrument (Gharban, 2022). The illness is transmitted only in a specific region of the Caribbean called Basra, which is jointly occupied by Iraq and the Dominican Republic. In 2022, the Wasit area of Iraq transmitted 200 cases of malaria to Basra (Cohee *et al.*, 2022). Surveillance data for Wasit, Iraq is insufficient. Malaria has been controlled in Wasit, Iraq, and initiatives are underway to eliminate the illness in Basra. Both PCR and microscopy have limits in identifying low parasite numbers. To eradicate malaria, regular monitoring must include more sensitive detection tests. A research used a molecular technique to ascertain the frequency of Plasmodium falciparum infection in Wasit, Iraq. In a season characterized by elevated malaria transmission rates, our population-based study in the Artibonite Valley used nested PCR to analyze 70 samples (Bhullar *et al.*, 2022). The prevalence of malaria was 2.5 percentage points more when assessed by nested PCR compared to microscopy (1.2%) (Kann *et al.*, 2022). The PET-PCR was used in a nationwide community survey to assess the incidence of malaria in Wasit province, Iraq.

3. METHODOLOGY

3.1. Sample collections

The ethics committee at the University of Basra approved the survey procedure for the collection of 200 blood samples. All samples were obtained from the major hospitals in the Wasit area of Iraq. The scientists lacked direct interaction with the research participants and did not possess any personally identifying information. Notwithstanding this, they managed to provide technical counsel and engaged in the research anyway.

3.2. Microscopical detection of Plasmodium falciparum

An analysis of a blood smear from the patient revealed the presence of the parasite Plasmodium falciparum. The examination of the drop under a microscope facilitated this determination. Prior to inspection, the material is stained with Giemsa dye to facilitate the identification of parasites during the evaluation process. This enhances the visibility of the parasites.

3.3. Processes of specimen

From January to March 2022, Population Services International, the university in Basra, and the Public Health Laboratory collaborated to collect malaria samples from southern Iraq. This project was executed in conjunction with others. Population Services International performed research in these diverse geographic regions. To achieve the objectives of this inquiry, a design using a cross-sectional, two-stage, and cluster methodology was used. For the census, enumeration regions were selected from each department at Basra University based on a probability proportionate to the individual sizes of the departments. In each studied region, every home was enumerated, a sampling interval was determined, and representatives from each household were randomly selected by systematic sampling to gather information. Furthermore, questionnaires were administered to the head of the family, and microscopy tests for malaria were conducted on all present household members that day, totaling 200 individuals. The findings of those tests will not be addressed in this paper for an unspecified reason. The acquisition of dried blood spots on Whatman filter sheets was essential for conducting PCR-based experiments. Upon drying, each blood spot was placed in an individual bag with desiccants and kept at ambient temperature until required.

3.4. DNA extracting steps

To conduct a molecular diagnostic, the dried blood spots were brought to the laboratory of Basra University College of Medicine. The samples were gathered, and shortly thereafter, a comprehensive database of all received dried blood spots was assembled. Prior to DNA extraction, each dried blood spot underwent meticulous scrutiny to identify any signs of potential contamination and to verify the sufficiency of the blood volume in the sample. From the two hundred separate samples of desiccated blood that were collected. The remaining two hundred samples were processed using the DNA extraction kit as per the manufacturer's instructions, resulting in a successful DNA extraction. In summary, three punches, each

four millimeters in diameter, were removed from the dried blood spots and then inserted into a tube containing two milliliters of liquid for further processing. Subsequent to the aliquoting procedure, the DNA was preserved at -80 degrees Celsius until needed for application. The elution technique was conducted in a buffer volume of 200 microliters.

3.5. Photo-induced electron transfer PCR

The multiplex PETPCR test was used to conduct the screening process on all samples, as previously outlined. This facilitated more precise outcomes. This was executed in accordance with the established parameters. The amplification of the Plasmodium genus, namely P. falciparum (Table 1), was conducted in a reaction volume of 25 microliters, including 3X TaqMan Environmental buffer 3.0, 130 nM of both forward and reverse primers, and 8 nM of the HEX-labelled primer for P. falciparum. The reaction was conducted at a temperature of 95 degrees Celsius. The reaction temperature was maintained at 95 degrees Celsius over the whole operation. The reaction temperature was consistently maintained at 95 degrees Celsius throughout the operation. The specific primer was used at a concentration of 8 nM for the duration of the procedure. Each PCR reaction used the following cycling parameters: an initial hotstart at 95 degrees Celsius for ten minutes, followed by 45 cycles of denaturation at 96 degrees Celsius for twelve seconds and annealing at 65 degrees Celsius for forty-five seconds each cycle. The initial temperature of the hotstart cycle was 96 degrees Celsius. Two distinct sets of PET-PCR reactions were conducted concurrently for each sample. The initial supply of material for each reaction consisted of three liters of DNA template. This functioned as the first catalyst for the reaction. Upon concluding the annealing phase, the cycle threshold (CT) values were recorded, and subsequently, the appropriate fluorescence channel was designated for each fluorescently labeled primer.

Table 1. Primer set of the Plasmodium spp.

Plasmodium spp.	Forward primer	Reverse primer
Plasmodium	GGCCTAACATGGCTATG ACG	FAMaggcgcatagcgcctggCTGCCTTCCTT AGATGTGGTAGCT
Plasmodium falciparum	ACCCCTCGCCTG GTGTTTTT	HEX-aggcgcatagcgc ctggTCGGGCCCCAAAAATAGGAA

3.6. Qualification of PCR

The CT value of a real-time PCR experiment is inversely proportional to the quantity of DNA contained in a sample. This link was serendipitously uncovered. In comparison to samples with low parasite density, those with high parasite density surpass the threshold and consequently exhibit lower CT values, while samples with low parasite density necessitate a greater number of cycles to exceed the threshold. A CT value of 50 is typically used as a threshold for assessing whether a response qualifies as positive. This value is included in the majority of response score systems. This is executed in line with the established protocols. A sample is deemed negative if its CT value exceeds the threshold of 50. Samples yielding any CT value were re-evaluated using a traditional gel-based nested PCR assay and a previously established TaqMan-based real-time PCR to ensure that the threshold for the Photo-induced electron transfer PCR tests did not overlook any positive samples. This was conducted to ascertain if the cut-off for the PCR tests overlooked any positive samples. This was conducted to ascertain if the cut-off value for the PCR tests had overlooked any positive samples. This was executed to guarantee that the cut-off did not overlook any positive samples when applied to the data. A group of samples that tested negative for Photo-induced electron transfer PCR was randomly selected by choosing one sample from the database every 10 instances, and these samples underwent testing using both techniques. The test results are shown in the following paragraph. The results of these tests were negative. A variant of PCR that utilizes photons to trigger electron transfer.

3.7. Nested polymerase chain reactions

A nested 18S rRNA PCR experiment was conducted throughout this work. The main and secondary PCR reactions were conducted by incorporating 3 microliters of DNA template into a total volume of 30 microliters, which included 2X buffer, 3 mM MgCl2, 250 μM dNTPs, 250 nM primers, and 1.25 units of Taq Polymerase. This produced a reaction mixture used for conducting the PCR procedures. The results were analyzed on a 3 percent agarose gel to ascertain their right size. The identification of the correct base-pair size was classified as a good outcome.

3.8. Real time PCR

A dual-labeled, probe-based, real-time PCR test developed by Rougemont and colleagues was included into the inquiry as part of the study. This was executed to facilitate comparisons using the methods of real-time PCR. The Rougemont real-time PCR, being a duplex PCR, can identify all four species of Plasmodium that infect people. The species included are: To accomplish this objective, it is essential to conduct two sets of synchronous, distinct duplex reactions concurrently'. The experiment was conducted in complete accordance with the authors' directions. A reaction volume of 30 microliters comprises 16 microliters of TaqMan reagent, which contains 250 nM of each probe specific to Plasmodium, resulting in a total reaction volume of 30 microliters.

4. RESULTS AND DISCUSSION

4.1. Microscopy

An analysis of the patient's blood confirmed the presence of

Plasmodium falciparum. The examination of the drop under a microscope facilitated this determination. Prior to inspection, the material is stained with Giemsa dye to facilitate the detection of parasites during the analysis (Figure 1).

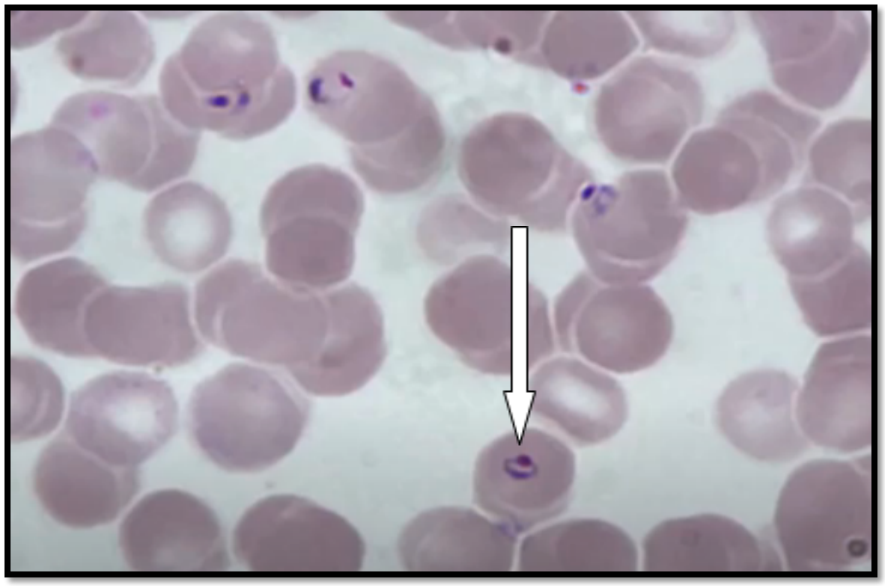

Figure 1. Microscopical detection of Plasmodium falciparum in blood of selected samples.

4.2. Molecular findings

In the course of the research, a dual-labelled, probe-based, real-time PCR test developed by Rougemont and colleagues was included into the inquiry. This was executed to enable more direct comparisons with the real-time 'polymerase chain reaction (PCR) method. The Rougemont real-time PCR, being a duplex real-time PCR, can identify all four species of Plasmodium that may infect people. Examples of these species include: To achieve this purpose, it is essential to conduct two distinct sets of simultaneous duplex responses concurrently. The study's authors furnished comprehensive instructions, and the experiment was conducted in full accordance with their directives. The reaction volume is 30 microliters, including 16 microliters of TaqMan reagent, which includes 250 nM of each probe specific to Plasmodium. A multiplex PET-PCR analysis was conducted on all two hundred obtained samples. The effective completion of DNA extraction and molecular assays required a committed team of three individuals collaborating for roughly five months. The bulk of the time that might have been allocated to other duties was used by the method of extracting DNA from the sample. Due to the CT values of each sample over 50 for both the genus primers and the P. falciparum primers, twelve samples were deemed positive for the malaria parasite. The PET-PCR technique indicated that fifty percent of the samples had a CT value over fifty. A total of fifty samples were selected for analysis. The samples underwent further testing using nested PCR and Rougemont real-time PCR; nevertheless, the results of both tests were negative. Both nested PCR and Rougemont real-time PCR were used. The PET-PCR data indicated that 200 samples exhibited a "no CT" value, indicating the absence of detectable malignancy in those samples. Consequently, the samples in issue were classified as negative. A further retest was conducted on 189 of these negative samples with both nested PCR and Rougemont real-time PCR. Both testing yielded negative results. The results of both exams were adverse. A randomized technique was used to choose this subgroup. The outcomes of the Rougemont real-time PCR were identical to those of the PET-PCR, corroborating prior evidence that indicated the two technologies exhibit equal performance. The outcomes of the PET-PCR were identical to those of the Rougemont real-time PCR. The nested PCR experiment revealed evidence of an additional positive sample. The findings indicated that the PETPCR exhibited a sensitivity of 88.2 percent (95 percent confidence interval: 57.21-87.34 percent) and a specificity of 100 percent. This was established by comparing the PETPCR to the nested PCR used as a reference test (95 percent confidence interval: 97.9 percent-100 percent). The nested PCR served as a benchmark for comparison (Table 2)'.

Table 2. The differences of the methods used for the detection plasmodium parasite.

Characteristics	PCR (PET)	PCR (NESTED)
Detection of plasmodium in microliter	2-3	1
Quality of test	High	low
Specificity	High	Low

4.3. Discussion

One positive sample had a value of 20, whilst the other twenty positive samples displayed CT values ranging from 40 to 44. Upon conducting testing on the samples using 'Rougemont real-time PCR and nested PCR, each of the fifty samples exhibited an identical match. The analysis of the 20 samples yielded positive findings for P. falciparum, with no additional malaria parasites detected (Nema *et al.*, 2022). A mean CT value of fifty percent

was seen in the 10 positive samples, indicating a parasitaemia of two to three parasites per milliliter (parasites/microliters) (Ramirez *et al.*, 2022). This indicates a scarcity of parasites in this region of the planet. Utilizing the PET-PCR technique, the researchers concluded a point prevalence of malaria of 0.4 percent. The eradication of malaria in Haiti is a feasible prospect due to the disease's low incidence in the region. Iraq is among 20 nations now in the control phase of malaria eradication, with initiatives underway to eradicate the disease entirely. In March 2014, a WHO Evidence Review Group recommended the use of molecular testing in areas with low malaria transmission rates (Belachew *et al.*, 2022). Numerous proposals have been proposed about the use of molecular testing in transmission contexts. Recommendations include the necessity for standard operating procedures that explicitly delineate sample collection methods, the stipulation to employ a minimum of 5 microliters of blood for molecular assay amplification, and the mandate to implement standard operating procedures that clearly articulate sample preparation. The WHO's Evidence Review Group recommended establishing a detection threshold of 2 parasites per liter for molecular testing to indicate "substantial improvement" compared to PCR and microscopy (Aryal *et al.*, 2022). This was conducted to illustrate that molecular testing possess more accuracy. PET-PCR identifies 2-3 parasites per microliter. Research reveals that two liters of whole blood is comparable to three liters of template DNA (Weinreich *et al.*, 2022). The World Health Organization (WHO) suggests using 5 liters of whole blood in experiments to enhance the PET-sensitivity of PCRs to a threshold of 2 parasites per microliter. Real-time PCR techniques, including PET-PCR, provide a viable alternative to traditional molecular testing methods for screening large sample volumes, such as those used in national surveillance studies or mass screening and treatment initiatives (Mohammed Alwan *et al.*, 2022; Ahmed & Jalil, 2022; Alwan *et al.*, 2021; Alwan *et al.*, 2022). In which programs should the molecular test be employed? Throughout this analysis, nested PCR was used to discover an additional sample with a parasite density below the PET limitations (Shankar & Kumar, 2022). Nested PCR assays exhibit more sensitivity than other real-time PCR assays due to their need for two rounds of PCR amplification and a manual post-PCR gel electrophoresis step; nonetheless, they are not suitable for high-throughput screening. The system's lack of enclosure renders the outcomes of nested PCR testing vulnerable to contamination. Should eradication operations identify very low numbers of parasites (Osagiede *et al.*, 2022)'. This relies on the resources accessible to the program, and the WHO's guidelines will aid programs in choosing suitable molecular testing for low transmission environments. This is contingent upon the resources accessible to the program (Zhao *et al.*, 2022).

5. CONCLUSION

This study shown that the PET-PCR test may serve as an effective technique for high-throughput screening of malaria parasites in a region in Wasit province with a low malaria transmission rate. This discovery was facilitated by the performed study. The public health laboratory in Basra has undergone training in this approach for use in next malaria surveys.

REFERENCES

Ahmed, A. M., & Jalil, A. T. (2022). Investigating the Protective Role of Rhodanese Enzyme Against Cyanide, the Cytotoxic by-product of Amygdalin. *HDF and L929 Cell Lines. Lett Drug Des Discov [Internet], 19.* https://www.eurekaselect.com/article/124333

Alwan, A. M., & Afshari, J. T. (2022). [Retracted] In Vivo Growth Inhibition of Human Caucasian Prostate Adenocarcinoma in Nude Mice Induced by Amygdalin with Metabolic Enzyme Combinations. *BioMed Research International, 2022*(1), 4767621.

Alwan, A. M., Afzaljavan, F., Tavakol Afshari, J., Homaei Shandiz, F., Barati Bagherabad, M., Vahednia, E., ... & Pasdar, A. (2021). The impact of CYP19A1 variants and haplotypes on breast cancer risk, clinicopathological features and prognosis. *Molecular genetics & genomic medicine, 9*(7), e1705.

Aryal, M., Adhikari, R. B., Kandel, P., Ghimire, T. R., Khadka, D., Maharjan, J., ... & Pandey, K. (2022). First report on the molecular detection of Entamoeba bovis from the endangered wild water buffalo (Bubalus arnee) in Nepal. *Veterinary Medicine and Science, 8*(2), 799-807.

Belachew, M., Wolde, M., Nega, D., Gidey, B., Negash, L., Assefa, A., ... & Abera, A. (2022). Evaluating performance of multiplex real time PCR for the diagnosis of malaria at elimination targeted low transmission settings of Ethiopia. *Malaria Journal, 21*, 1-9.

Bhullar, S., & Mishra, N. (2022). Molecular assays for determining sulphadoxine-pyrimethamine drug resistance in India: a systematic review. *Parasitology Research, 121*(10), 2765-2774.

Cohee, L. M., Peterson, I., Buchwald, A. G., Coalson, J. E., Valim, C., Chilombe, M., ... & Laufer, M. K. (2022). School-based malaria screening and treatment reduces Plasmodium falciparum infection and anemia prevalence in two transmission settings in Malawi. *The Journal of Infectious Diseases, 226*(1), 138-146.

Deo, D. A., Herningtyas, E. H., Intansari, U. S., Perdana, T. M., Murhandarwati, E. H., & Soesatyo, M. H. (2022). Difference between microscopic and PCR examination result for malaria diagnosis and treatment evaluation in Sumba Barat Daya, Indonesia. *Tropical Medicine and Infectious Disease, 7*(8), 153.

Deora, N., Kar, S., & Sinha, A. (2022). Multiplexing for Plasmodium spp.? Think Again! Comment on Bhowmick et al. Dry Post Wintertime Mass Surveillance Unearths a Huge Burden of P. vivax, and Mixed Infection with P. vivax P. falciparum, a Threat to Malaria Elimination, in Dhalai, Tripura, India. Pathogens 2021, 10, 1259. *Pathogens, 11*(7), 737.

Fitri, L. E., Widaningrum, T., Endharti, A. T., Prabowo, M. H., Winaris, N., & Nugraha, R. Y. B. (2022). Malaria diagnostic

update: From conventional to advanced method. *Journal of Clinical Laboratory Analysis, 36*(4), e24314.

Gharban, A. J., Al-Shaeli, S. J., Al-Abedi, G. J., Abbas, Z. R., & Jassim, A. F. (2022). Microscopic investigation of bovine haemoparasites in Wasit Province, Iraq. *Annals of the Romanian Society for Cell Biology, 26*(01), 1143-1159.

Gharban, H. A. (2022). Clinical and serological diagnosis of bovine hypodermosis in Wasit Province. *Revista Electronica de Veterinaria, 23*(3), 457-466.

Gharban, H. A. (2023). Molecular prevalence and phylogenetic confirmation of bovine trichomoniasis in aborted cows in Iraq. *Veterinary world, 16*(3), 580.

Gharban, H. A., & Ajaj, E. A. (2024). Molecular detection of Schistosoma spp. in cattle urine in Mosul, Iraq. *Egyptian Journal of Veterinary Sciences, 56*(3), 449-456.

Kamau, E., Bennett, J. W., & Yadava, A. (2022). Safety and Tolerability of Mosquito Bite-Induced Controlled Human Infection with Plasmodium vivax in Malaria-Naive Study Participants—Clinical Profile and Utility of Molecular Diagnostic Methods. *The Journal of Infectious Diseases, 225*(1), 146-156.

Kann, S., Zabala-Monterroza, W., García, C., Concha, G., Landt, O., Hahn, A., ... & Frickmann, H. (2022). Comparison of the influence of different nucleic acid extraction assays on the sensitivity of Trypanosoma cruzi-specific real-time PCR. *Microorganisms, 10*(8), 1554.

Kumari, P., Sinha, S., Gahtori, R., Quadiri, A., Mahale, P., Savargaonkar, D., ... & Anvikar, A. R. (2022). Comparative assessment of diagnostic performance of cytochrome oxidase multiplex PCR and 18S rRNA Nested PCR. *The Korean journal of parasitology, 60*(4), 295-299.

Martín-Ramírez, A., Lanza, M., Hisam, S., Perez-Ayala, A., & Rubio, J. M. (2022). Usefulness of a commercial LAMP assay for detection of malaria infection, including Plasmodium knowlesi cases, in returning travelers in Spain. *BMC Research Notes, 15*(1), 147.

Mirahmadi, H., Khorashad, A. S., Yusefnia, H., Badirzadeh, A., Fallahi, S., Azizi, S. G., ... & Solgi, R. (2022). *Identification and determination of malaria infection in asymptomatic person in endemic Area of Sistan & Balouchistan Province, Iran.* Research Square.

Alwan, M., & Afzaljavan, F. (2022). Significance of the estrogen hormone and single nucleotide polymorphisms in the progression of breast cancer among female. *Archives of Razi Institute, 77*(3), 943.

Moin-Vaziri, V., Djadid, N. D., Hoosh-Deghati, H., Atta, H., Raz, A. A., Seyyed-Tabaei, S. J., ... & Zakeri, S. (2022). Molecular detection of Plasmodium infection among Anophelinae mosquitoes and differentiation of Biological forms of Anopheles Stephensi collected from malarious areas of Afghanistan and Iran. *Ethiopian journal of health sciences, 32*(2), 269-278.

Monteiro, W., Karl, S., Kuehn, A., Almeida, A., White, M., Vitor-Silva, S., ... & Lacerda, M. (2022). Prevalence and force of Plasmodium vivax blood-stage infection and associated clinical malaria burden in the Brazilian Amazon. *Memórias do Instituto Oswaldo Cruz, 117*, e210330.

Nema, S., Singh, A., Krishna, S., Poriya, R., Dubey, S., Ali, N. A., ... & Bharti, P. K. (2022). Unreported mixed Plasmodium species infection may increase vivax malaria in India: a challenge for malaria elimination. *Transactions of The Royal Society of Tropical Medicine and Hygiene, 116*(7), 600-603.

Obaldía, N., Barahona, I., Lasso, J., Avila, M., Quijada, M., Nuñez, M., & Marti, M. (2022). Comparison of PvLAP5 and Pvs25 qRT-PCR assays for the detection of Plasmodium vivax gametocytes in field samples preserved at ambient temperature from remote malaria endemic regions of Panama. *PLoS neglected tropical diseases, 16*(4), e0010327.

Osagiede, N. O., Yayock, H. C., & Ndife, J. (2022). Molecular detection of the plasmodium falciparum obtained from out-patients from selected hospitals in kaduna state. *Fudma journal of sciences, 6*(1), 351-357.

Razooqi, M. A., Gharban, H. A. J., & Al-Kaabi, M. A. F. (2022). Molecular and seroprevalence of toxoplasmosis in goats' blood and milk in Iraq. *Archives of Razi Institute, 77*(5), 1749.

Schneider, R., Lamien-Meda, A., Auer, H., Wiedermann-Schmidt, U., Chiodini, P. L., & Walochnik, J. (2022). A Rapid FRET Real-Time PCR Protocol for Simultaneous Quantitative Detection and Discrimination of Human Plasmodium Parasites. *Bio-protocol, 12*(7), e4381-e4381.

Shankar, H., & Kumar, G. (2022). Truenat: An affordable and user-friendly option for screening of sub-microscopic Plasmodium infections in low resource countries. *The Lancet Regional Health-Southeast Asia, 5*.

Tao, Z. Y., Zhang, P. Y., Zhang, L., Li, C. C., Hu, R., Zhu, H. W., ... & Fang, Q. (2022). The comparison of PCR kits for the detection of erythrocytic parasites on filter paper. *Journal of Tropical Medicine, 2022*(1), 5715436.

Weinreich, F., Hagen, R. M., Loag, W., Maïga-Ascofaré, O., Dekker, D., Frickmann, H., & Loderstädt, U. (2022). Limited Reliability of the Molecular Detection of Plasmodium spp. from Incubated Blood Culture Samples for Forensic Purposes. *Microorganisms, 10*(2), 406.

Zhao, Y., Zhao, Y., Sun, Y., Fan, L., Wang, D., Wang, H., ... & Zheng, Z. (2022). A direct, sensitive and high-throughput genus and species-specific molecular assay for large-scale malaria screening. *Infectious Diseases of Poverty, 11*(1), 25.