Ann. For. Res. 66(1): 1969-1979, 2023
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
www.e-afr.org

MOLECULAR DETECTION OF MALARIA IN PATIENT OF EAST-SOUTHERN IRAQ

Thuraya khaled Abdulwahed
Affiliations: Nursing Department, Kut University College
Correspondence: Al kut, Wasit, Iraq, 52001
Thuraya.khaled@alkutcollege.edu.iq

Abstract

Introduction: Recent research resulted in the development of electron transfer PCR, which employs self-quenching primers to detect different species of *Plasmodium*. There was some discussion about *Plasmodium falciparum*. The PCR assay is a reliable and user-friendly alternative to real-time PCR methods. The possibility of using polymerase chain reaction (PCR) for the molecular detection of malaria parasites in east southern Iraq.
Material and Methods: DNA was isolated by QIAGEN from blood spots that had been dried out. On each of the 200 samples, PCR analysis was performed twice. Positive samples had a CT that was less than or equal to 60. Using nested PCR, 50 samples were validated for accuracy. In addition to that, a real-time PCR method based on TaqMan was used on these samples.
Findings: PCR was able to identify 20 positive samples out of a total of 200. These samples were found to be positive using both nested and TaqMan-based methods. Neither PCR nor TaqMan were successful in identifying a positive sample within a subset of 50, but nested PCR was.
Conclusion: Nested PCR is superior to PET-PCR in terms of sensitivity, but it is not the best choice for high-throughput sample screening. Nested PCR is a useful alternative assay for the rapid screening of a large number of samples in the laboratory because it is simple to implement and relatively inexpensive.
Keywords: Malaria, molecular, east-southern, PCR, *Plasmodium*.

Introduction

In order to measure the efficacy of intervention strategies and determine how resources should be distributed, the prevalence and distribution of malaria infection in a country can be used(1). The prevalence of the malaria parasite needs to be measured accurately(2). In order to identify malaria parasites, microscopy and other rapid diagnostic tests are utilized in a significant number of epidemiological surveillance studies(3). Following a recommendation from the WHO to delay treatment until after a parasitological diagnosis, an increase in the use of RDTs occurred. RDTs are simple to use, don't require electricity or specialized knowledge, and provide results that can be used to make decisions about treatment(4). A low-density parasite infection that is asymptomatic cannot be detected using microscopy(5). Malaria infections that are asymptomatic can act as transmission foci in both low transmission and high transmission settings, leading to new cases of infection(6). Detection of both symptomatic and asymptomatic parasite infections is necessary for malaria eradication programs(7). In comparison to microscopy and RDTs, molecular

tests such as PCR-based assays have a higher sensitivity and specificity, meaning that they can detect parasitaemia at levels as low as 2 parasites per liter(8). Microscopy and PCR on the other hand, produce lower estimates of the prevalence of malaria parasites than do epidemiological studies that use molecular testing(9). Molecular assays in large epidemiological surveillance studies face some challenges(10). Molecular tests are simple to carry out and don't cost very much money(11). A reliable molecular assay can screen a large number of samples efficiently and at a low cost. As opposed to traditional nested 18S rRNA PCR assays, realtime PCR assays are superior for use in large-scale research projects(12). The cost of many different types of tests is going down because reaction volumes are being made smaller and cheaper primers and fluorophores are being designed. The multiplexed photo-induced electron transfer PCR test is a reliable and cost-efficient diagnostic tool(13). Transmission of the disease is confined to a single area in the Caribbean known as Basra, which is shared by Iraq and the Dominican Republic. The east-southern region of Iraq was responsible for the transmission of 200 cases of malaria to Basra in 2022(14). There is a lack of surveillance data for east and southern Iraq. Malaria has been brought under control in east and southern Iraq, and efforts are currently being made to eradicate the disease from Basra. Both PCR and microscopy have their limitations when it comes to detecting low parasite densities. To eliminate malaria, routine surveillance must include more sensitive detection assays. In order to determine the prevalence of *Plasmodium falciparum* infection in southeast Iraq, one study employed a molecular assay. During a time of year with a high prevalence of malaria transmission, this population-based survey in the Artibonite Valley used nested PCR to test 70 samples(15). The prevalence of malaria was 2.5 percentage points higher when measured by nested PCR than when measured by microscopy (1.2 percent)(16). In this particular instance, the PET-PCR was utilized in the context of a national community survey in order to estimate the prevalence of malaria in east southern Iraq.

Material and methods

Sample collections

The ethical committee from the university of Basra gave their stamp of approval to the survey protocol for the 200 blood samples. All of the samples were collected from the primary hospitals in the east and southern regions of Iraq. The investigators did not have any direct contact with the people who participated in the study, nor did they have access to any personally identifiable information. Despite this, they were able to provide technical advice and participated in the study all the same.

Microscopical detection of *Plasmodium falciparum*

An examination of a drop of the patient's blood that had been spread out as a blood smear on a microscope slide allowed for the discovery of the parasite *Plasmodium falciparum*. Examining the drop under a microscope allowed for this to be determined. Before the specimen is examined, it is stained with the Giemsa stain so that the parasites can be more easily identified during the process of examination. This helps the parasites to stand out more clearly.

Processes of specimen

During the months of January through March of 2022, Population Services International, the university in Basra, and the Public Health Laboratory worked together in an effort to collect malaria samples from southern regions of Iraq. This project was carried out in collaboration with one another. Research was conducted by Population Services International in these various geographic locations. In order to accomplish the goals of this investigation, a design comprising a cross-sectional, two-stage, and cluster approach was utilized. For the purpose of the census, enumeration areas were chosen from each of the departments at Basra University utilizing a probability that was proportional to the sizes of the departments individually. Within each area that was sampled, every household was cataloged, a sampling interval was established, and using systematic sampling, representatives from each household were chosen at random as a means of collecting information. In addition to this, questionnaires were given to the head of the household, and microscopy tests for malaria were performed on every member of the household who was present on that particular day (a total of 200 people). In contrast, the results of those tests will not be discussed in this manuscript for some unknown reason. The collection of dried blood spots on Whatman filter papers was necessary in order to carry out PCR-based assays. After being dried, these blood spots were each put into their own bag, along with desiccants, and stored at room temperature until they were needed.

DNA extracting steps

In order to carry out a molecular diagnosis, the blood spots that had been allowed to dry out were transported to the laboratories of the Basra University College of Medicine. The samples were collected, and almost immediately afterward, a complete database of all of the dried blood spots that were received was compiled. Before the DNA was extracted, every spot of dried blood was given a careful examination to look for any indications of possible contamination and to check that there was an adequate amount of blood in the sample. From among the two hundred individual spots of dried blood that were obtained. The remaining two hundred samples were subjected to the DNA extracting kit's processing according to the instructions provided by the manufacturer, and a successful extraction of DNA was accomplished. To provide a brief summary, three punches, each measuring four millimeters, were extracted from the dried blood spots and then placed, as instructed, into a tube that contained two milliliters of liquid for the purpose of further processing. Following the aliquoting process, the DNA was stored at a temperature of -80 degrees Celsius until it was required for use. The elution procedure was carried out in a buffer volume measuring 200 microliters.

Photo-induced electron transfer PCR

In order to carry out the screening procedure on each and every sample, as was previously described, the multiplex PETPCR assay was used. This allowed for more accurate results. This was carried out in a manner that is in accordance with the guidelines that were set up. In a nutshell, the amplification of the *Plasmodium* genus or *P. falciparum* (Table 1) was performed in a reaction volume of 25 microliters containing 3X TaqMan Environmental buffer 3.0, 130 nM of forward and reverse primers, and 8 nM of the P. falciparum HEX-labelled primer. The reaction was carried

out at a temperature of 95 degrees Celsius. The temperature of the reaction was maintained at 95 degrees Celsius throughout the process. Throughout the entirety of the process, the temperature of the reaction was held steady at 95 degrees Celsius. The particular primer in question was applied at a concentration of 8 nM throughout the entirety of the process that was being carried out. For each individual PCR reaction, the following cycling parameters were utilized: 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 in each cycle. The temperature at the start of the hotstart cycle was 96 degrees Celsius. For each sample, two different sets of PET-PCR reactions were carried out simultaneously. The first source of material for each reaction was three liters' worth of DNA template. This served as the starting point for the reaction. Following the completion of the annealing step, the cycle threshold CT values were noted down, and after that, the correct fluorescence channel was selected for each fluorescently labelled 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

Qualification of PCR

It has been demonstrated that the CT value of an assay that is performed using real-time PCR is inversely proportional to the amount of DNA that is present within a sample. This relationship was discovered by accident. When compared to samples that contain a low parasite density, those that contain a high parasite density are able to pass the threshold and, as a direct result of this, have lower CT values, whereas samples that contain a low parasite density require a greater number of cycles in order to be able to pass the threshold. It is customary to use a CT value of 50 as a cutoff point when determining whether or not a reaction should be scored as positive. This value can be found in most reaction scoring systems. This is carried out in accordance with the predetermined procedures. As a direct result of this, a sample is considered to be negative if it possesses a CT value that is higher than the threshold value of 50. Samples that produced any CT value were retested using a conventional gel-based nested PCR assay as well as a previously described TaqMan-based real-time PCR in order to verify that the cut-off for the Photo-induced electron transfer PCR assays did not miss any positive samples. This was done in order to determine whether or not the cut-off for the PCR assays missed any positive samples. This was done so that it could be determined whether or not the cut-off value for the PCR assays had missed any positive samples. This was done to ensure that the cut-off did not miss any positive samples when it was applied to the data. In addition, a subset of samples that were negative for Photo-induced electron transfer PCR were chosen at random by selecting one sample from the database every ten times, and these samples were put through the tests using both of these methods. The results of the tests

can be found in the following paragraph. The outcomes of these examinations came back negative. A form of PCR that employs photons as an electron transfer inducer.

Nested polymerase chain reactions

Throughout the course of this investigation, a nested 18S rRNA PCR assay was carried out. In a nutshell, the primary and secondary PCR reactions were performed by adding 3 microliters of DNA template to a total volume of 30 L that also contained 2X buffer, 3 mM MgCl2, 250 M dNTPs, 250 nM primers, and 1.25 units of Taq Polymerase. This created a reaction mixture that was used to carry out the PCR reactions. On an agarose gel that contained 3 percent agarose, the products were examined to determine if they were the correct size. The detection of the appropriate base-pair size was defined as a positive result.

Real time PCR

A dual-labelled, probe-based, real-time PCR assay that had been developed by Rougemont and colleagues was incorporated into the investigation as part of the study. This was done so that comparisons with the methodology of real-time PCR could be made in an easier manner. The fact that the Rougemont real-time PCR is a duplex PCR enables it to detect all four species of Plasmodium that are capable of infecting humans. These species include: In order to achieve this goal, it is necessary to carry out two sets of simultaneous separate duplex reactions simultaneously. This experiment was carried out in a manner that was entirely consistent with the instructions provided by the authors. A reaction volume of 30 microliters containing 16 microliters of TaqMan reagent containing 250 nM of each of the probes that are specific to Plasmodium; the volume of the reaction is totaled as 30 microliters.

Results

Microscopical appearance of *Plasmodium falciparum*

An examination of the patient's blood that had been indicated presence *of Plasmodium falciparum*. Examining the drop under a microscope allowed for this to be determined. Before the specimen is examined, it is stained with the Giemsa stain so that the parasites were detected during the process of examination (Figure 1).

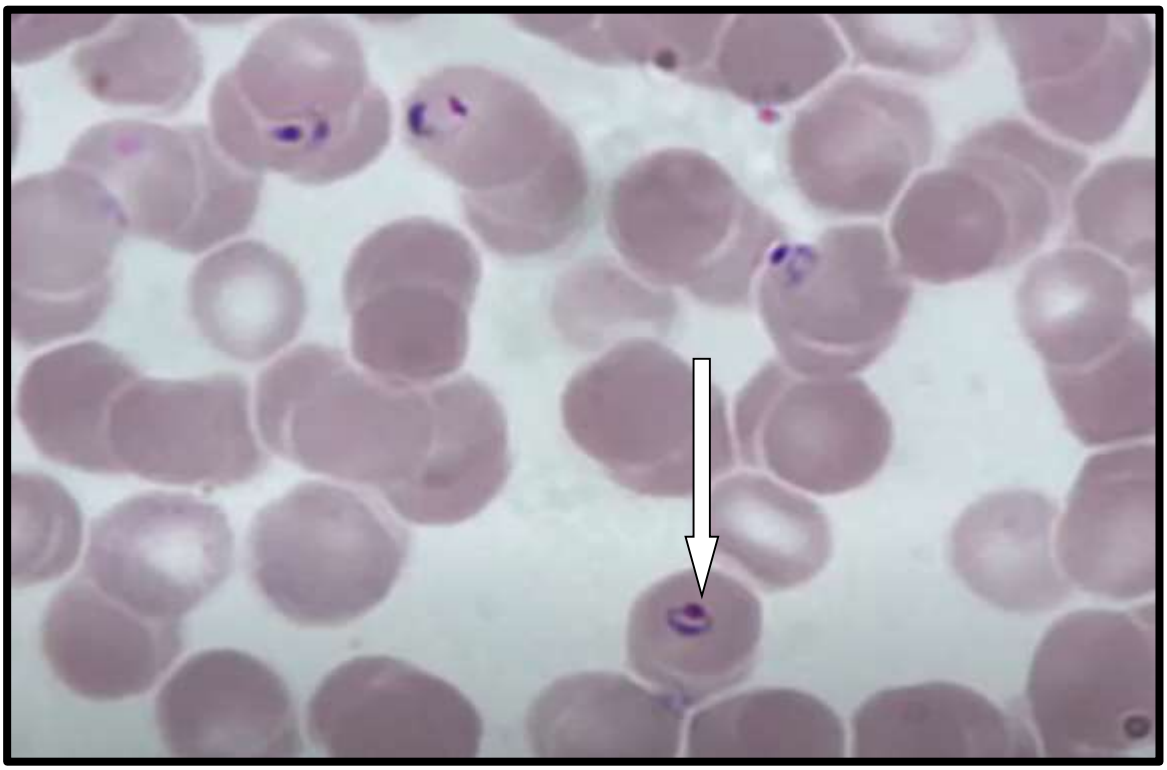

Figure 1: microscopical detection of *Plasmodium falciparum* in blood of selected samples.

Molecular findings

As part of the investigation that was conducted as a part of the study, a dual-labelled, probe-based, real-time PCR assay that had been developed by Rougemont and colleagues was incorporated into the investigation. This was done in order to facilitate more straightforward comparisons with the procedure of real-time polymerase chain reaction (PCR). Because it is a duplex real-time PCR, the Rougemont real-time PCR can detect all four species of Plasmodium that are capable of infecting humans. Some examples of these species are: In order to accomplish this objective, it is necessary to carry out two different sets of simultaneous duplex reactions at the same time. The authors of this study provided detailed instructions, and the experiment was carried out in a way that was completely compliant with those instructions. The total volume of the reaction is 30 microliters, and it contains 16 microliters of TaqMan reagent, which contains 250 nM of each of the probes that are specific to Plasmodium. A multiplex PET-PCR analysis was carried out on each and every one of the two hundred samples that were collected. In order to successfully complete the process of DNA extraction and the molecular tests, it required a dedicated team of three people working together for approximately five months. The majority of the time that could have been spent on other tasks was eaten up by the procedure that consisted of extracting the DNA from the sample. As a result of the fact that the CT values of each of those samples were greater than 50 for the genus primers as well as the P. falciparum primers, a total of twelve of the samples were considered to be positive for the presence of the malaria parasite. It was demonstrated by the PET-PCR method that fifty percent of the samples had a CT value that was higher than fifty. Overall, fifty of the samples were considered for the analysis. These samples were put through additional testing with

nested PCR and Rougemont real-time PCR; however, the results of both sets of tests came back negative. nested PCR and Rougemont real-time PCR were both used. It was determined, on the basis of the findings of the PET-PCR, that a total of 200 of the samples had a value of "no CT," meaning that there was no detectable amount of cancer in those samples. As a direct result of this discovery, the samples in question were labeled as negative. After that, a subsequent retest was carried out on 189 of these negative samples making use of both the nested PCR and the Rougemont real-time PCR. Both of these tests came back negative. The outcomes of both of these examinations were unfavorable. A randomization method was utilized in order to select this subset. The results of the Rougemont real-time PCR were the same as those of the PET-PCR, which is consistent with a previous demonstration that demonstrated that the two methods had equivalent performance. The results of the PET-PCR were the same as those of the Rougemont real-time PCR. On the other hand, the nested PCR assay discovered indications that there was an additional positive sample present. The results showed that the PETPCR had a sensitivity of 88.2 percent (95 percent confidence interval: 57.21-87.34 percent), and that it had a specificity of 100 percent. This was determined by comparing the PETPCR to the nested PCR that was used as a reference test (95 percent confidence interval: 97.9 percent-100 percent). The nested PCR was utilized as a standard for comparison (Table 2).

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

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

Discussion

The remaining positive sample had a value of 20, while the other twenty positive samples had CT values that ranged from 40 to 44. After running the samples through tests with Rougemont real-time PCR and nested PCR, every single one of the fifty samples was found to be an exact match. The tests conducted on the 20 samples returned positive results for the presence of *P. falciparum*, but no other malaria parasites were identified(17). A mean CT value of fifty percent was found in the ten positive samples, which indicated a parasitaemia of two to three parasites per milliliter (parasites/ microliters)(18). This is evidence that there are not many parasites living in this part of the world. By employing the PET-PCR assay, the researchers were able to arrive at the conclusion that there was a point prevalence of malaria of 0.4 percent. Eradication of malaria is a distinct possibility in Haiti as a result of the disease's low prevalence there(19). Iraq is one of 20 countries that is currently in the control phase of the malaria elimination phase, and there are efforts being made to eliminate the disease completely(20). In March of 2014, a WHO Evidence Review Group proposed the utilization of molecular tests in regions of the world that have a low rate of malaria transmission(21). There have been a number of recommendations made in regards to the utilization of molecular tests in transmission settings. Some of these recommendations include the

requirement for standard operating procedures that clearly define sample collection methods, the requirement to use at least 5 microliters of blood for molecular assay amplification, and the requirement to utilize standard operating procedures that clearly define sample preparation(22). The Evidence Review Group of the WHO suggested that the detection limit for molecular tests be set at 2 parasites per liter in order to demonstrate "substantial improvement" over PCR and microscopy(23). This was done in order to demonstrate that molecular tests are more accurate. PET-PCR detects 2-3 parasites/ microliters. The research that has been done indicates that two liters of whole blood is equivalent to three liters of template DNA(24). In order to improve the PET-sensitivity PCRs to a level of 2 parasites/microliters, the World Health Organization (WHO) recommends using 5 liters of whole blood in the experiment. Real-time PCR assays, such as PET-PCR, offer a practical alternative to more conventional methods of conducting molecular testing when it comes to the screening of large numbers of samples, such as those used in national surveillance studies or mass screening-and-treatment programs(25–28). In which of the programs should the molecular test be utilized. During the course of this investigation, nesting PCR was used to identify an additional sample that contained a parasite density that was lower than the PET-limits(29). Because they require two rounds of PCR amplification and a step of manual post-PCR gel electrophoresis, nested PCR assays are more sensitive than the majority of real-time PCR assays; however, high throughput screening cannot be performed with them.(30) Because the system is not an enclosed one, the results of nested PCR tests are susceptible to contamination. Should elimination programs detect incredibly low densities of parasites(31). This is dependent on the resources that are available to the program, and the recommendations provided by the WHO will assist programs in selecting appropriate molecular tests for low transmission settings. This is dependent on the resources that are available to the program(32).

Conclusion

In an area of Iraq's east and south that has a low malaria transmission rate, this research was able to demonstrate that the PET-PCR assay can be useful as a tool for high throughput screening for malaria parasites. This was a finding that was made possible by the research that was conducted. The public health lab in Basra has received training in this methodology so that it can be utilized in the upcoming malaria surveys.

References

1. Obaldía N, Barahona I, Lasso J, Avila M, Quijada M, Nuñez M, et al. 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 Negl Trop Dis. 2022;16(4):e0010327.
2. Tao Z, Zhang P, Zhang L, Li C, Hu R, Zhu H, et al. The Comparison of PCR Kits for the Detection of Erythrocytic Parasites on Filter Paper. J Trop Med. 2022;2022.
3. Kamau E, Bennett JW, Yadava A. 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. J Infect Dis. 2022;225(1):146–56.

4. Martín-Ramírez A, Lanza M, Hisam S, Perez-Ayala A, Rubio JM. Usefulness of a commercial LAMP assay for detection of malaria infection, including Plasmodium knowlesi cases, in returning travelers in Spain. BMC Res Notes. 2022;15(1):1–6.
5. Pian H, Yang M, Sun X, Zheng Z. Sandwich hybridization-based loop-mediated isothermal amplification (SHB-LAMP) for high-throughput detection of malaria RNA from asymptomatic infections. Sensors Actuators B Chem. 2022;365:131973.
6. Pal-Bhowmick I, Nirmolia T, Pandey A, Subbarao SK, Nath A, Senapati S, et al. Reply to Deora et al. 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. 2022;11(8):832.
7. Moin-Vaziri V, Djadid ND, Hoosh-Deghati H, Atta H, Raz AA, Seyyed-Tabaei SJ, et al. Molecular Detection of Plasmodium Infection among Anophelinae Mosquitoes and Differentiation of Biological Forms of Anopheles Stephensi Collected from Malarious Areas of Afghanistan and Iran. Ethiop J Health Sci. 2022;32(2).
8. Fitri LE, Widaningrum T, Endharti AT, Prabowo MH, Winaris N, Nugraha RYB. Malaria diagnostic update: From conventional to advanced method. J Clin Lab Anal. 2022;36(4):e24314.
9. Deora N, Kar S, Sinha A. 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. 2022;11(7):737.
10. Costa GL, Alvarenga DAM, Aguiar ACC, Louzada J, Pereira DB, De Oliveira TF, et al. Improving the molecular diagnosis of malaria: droplet digital PCR-based method using saliva as a DNA source. Front Microbiol. 2022;13.
11. Kumari P, Sinha S, Gahtori R, Quadiri A, Mahale P, Savargaonkar D, et al. Comparative Assessment of Diagnostic Performance of Cytochrome Oxidase Multiplex PCR and 18S rRNA Nested PCR. Korean J Parasitol. 2022;60(4):295–9.
12. Basile AJ, Niedrig M, Lambert AJ, Meurant R, Brault AC, Domingo C, et al. Laboratory evaluation of RealStar Yellow Fever Virus RT-PCR kit 1.0 for potential use in the global yellow fever laboratory network. PLoS Negl Trop Dis. 2022;16(9):e0010770.
13. Rahi M, Sharma R, Saroha P, Chaturvedi R, Bharti PK, Sharma A. Polymerase Chain Reaction-Based Malaria Diagnosis Can Be Increasingly Adopted during Current Phase of Malaria Elimination in India. Am J Trop Med Hyg. 2022;
14. Cohee LM, Peterson I, Buchwald AG, Coalson JE, Valim C, Chilombe M, et al. School-based malaria screening and treatment reduces Plasmodium falciparum infection and anemia prevalence in two transmission settings in Malawi. J Infect Dis. 2022;
15. Bhullar S, Mishra N. Molecular assays for determining sulphadoxine-pyrimethamine drug resistance in India: a systematic review. Parasitol Res. 2022;1–10.
16. Kann S, Zabala-Monterroza W, García C, Concha G, Landt O, Hahn A, et al. Comparison

of the Influence of Different Nucleic Acid Extraction Assays on the Sensitivity of Trypanosoma cruzi-Specific Real-Time PCR. Microorganisms. 2022;10(8):1554.

17. Nema S, Singh A, Krishna S, Poriya R, Dubey S, Ali NA, et al. Unreported mixed Plasmodium species infection may increase vivax malaria in India: a challenge for malaria elimination. Trans R Soc Trop Med Hyg. 2022;
18. Ramírez AM, Suárez ML, García CM, Hisam SR, Perez-Ayala A, Rubio JM. Usefulness of Malachite-Green LAMP for Diagnosis of Plasmodium and Five Human Malaria Species in a Nonendemic Setting. Am J Trop Med Hyg. 2022;
19. Schneider R, Lamien-Meda A, Auer H, Wiedermann-Schmidt U, Chiodini PL, Walochnik J. A Rapid FRET Real-Time PCR Protocol for Simultaneous Quantitative Detection and Discrimination of Human Plasmodium Parasites. Bio-protocol. 2022;12(7):e4381–e4381.
20. Mirahmadi H, Khorashad AS, Yusefnia H, Badirzadeh A, Fallahi S, Azizi SG, et al. Identification and determination of malaria infection in asymptomatic person in endemic Area of Sistan & Balouchistan Province, Iran. 2022;
21. Belachew M, Wolde M, Nega D, Gidey B, Negash L, Assefa A, et al. Evaluating performance of multiplex real time PCR for the diagnosis of malaria at elimination targeted low transmission settings of Ethiopia. Malar J. 2022;21(1):1–9.
22. Monteiro W, Karl S, Kuehn A, Almeida A, White M, Vitor-Silva S, et al. Prevalence and force of Plasmodium vivax blood-stage infection and associated clinical malaria burden in the Brazilian Amazon. Mem Inst Oswaldo Cruz. 2022;117.
23. Aryal M, Adhikari RB, Kandel P, Ghimire TR, Khadka D, Maharjan J, et al. First report on the molecular detection of Entamoeba bovis from the endangered wild water buffalo (Bubalus arnee) in Nepal. Vet Med Sci. 2022;8(2):799–807.
24. Weinreich F, Hagen RM, Loag W, Maïga-Ascofaré O, Dekker D, Frickmann H, et al. Limited Reliability of the Molecular Detection of Plasmodium spp. from Incubated Blood Culture Samples for Forensic Purposes. Microorganisms. 2022;10(2):406.
25. Mohammed Alwan A, Tavakol Afshari J, Afzaljavan F. Significance of the Estrogen Hormone and Single Nucleotide Polymorphisms in the Progression of Breast Cancer among Female. Arch Razi Inst [Internet]. 2022;77(3):943–58. Available from: https://archrazi.areeo.ac.ir/article_126343.html
26. Ahmed AM, Jalil AT. Investigating the Protective Role of Rhodanese Enzyme Against Cyanide, the Cytotoxic by-product of Amygdalin, in HDF and L929 Cell Lines. Lett Drug Des Discov [Internet]. 2022;19. Available from: https://www.eurekaselect.com/article/124333
27. Alwan AM, Afzaljavan F, Tavakol Afshari J, Homaei Shandiz F, Barati Bagherabad M, Vahednia E, et al. The impact of CYP19A1 variants and haplotypes on breast cancer risk, clinicopathological features and prognosis. Mol Genet genomic Med. 2021;9(7):e1705.
28. Alwan AM, Afshari JT. In Vivo Growth Inhibition of Human Caucasian Prostate Adenocarcinoma in Nude Mice Induced by Amygdalin with Metabolic Enzyme Combinations. Biomed Res Int. 2022;2022.

29. Shankar H, Kumar G. Truenat: An affordable and user-friendly option for screening of sub-microscopic Plasmodium infections in low resource countries. Lancet Reg Heal Asia. 2022;5.
30. Deo DA, Herningtyas EH, Intansari US, Perdana TM, Murhandarwati EH, Soesatyo MHNE. Difference between Microscopic and PCR Examination Result for Malaria Diagnosis and Treatment Evaluation in Sumba Barat Daya, Indonesia. Trop Med Infect Dis. 2022;7(8):153.
31. Osagiede NO, Yayock HC, Ndife J. MOLECULAR DETECTION OF THE PLASMODIUM FALCIPARUM OBTAINED FROM OUT-PATIENTS FROM SELECTED HOSPITALS IN KADUNA STATE. FUDMA J Sci. 2022;6(1):351–7.
32. Zhao Y, Zhao Y, Sun Y, Fan L, Wang D, Wang H, et al. A direct, sensitive and high-throughput genus and species-specific molecular assay for large-scale malaria screening. Infect Dis poverty. 2022;11(1):1–12.