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Studying diversification of microbes in soil around industrial zones of Makori, Gurguri, Nashpa, district Karak, Khyber Pakhtunkhwa

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Malaria elimination strategies demand constant monitoring of the parasite population for genetic variations that necessitate a public health response, such as a new form of drug resistance. Malaria control relies mainly on rapid and precise diagnosis, followed by successful treatment. Malaria diagnosis must be made as soon as possible to provide optimal disease management and surveillance. In every situation, the accuracy of malaria diagnosis is essential, as misdiagnosis can result in severe morbidity and mortality. Here, we describe a novel, high through put method using an Illumina Mi-Seq platform to demonstrate the proportions of *Plasmodium* species in metabarcoded DNA samples derived from human malaria patients. We reported a unique, high throughput approach for determining the ratio of *Plasmodium* species in metabarcoding DNA samples generated from human malaria patients using an Illumina Mi-Seq. Positive control gDNA from *P. falciparum* and *P. vivax* was used to mock DNA pols of parasites to test the assay direction threshold for each species. The several mock pools indicate the accuracy of the detection abilities and the proportion of each species present. The technique was subsequently used on malaria-positive patient samples to determine the species composition of *Plasmodium* populations in Pakistan's Punjab region and the tribal territories of the Auragzai Agency Border (ShanaweriZargiri) Ali Masjid Landi-Kotal Khyber Agency border the Pakistan-Afghanistan. The deep amplicon sequencing approach contrasts with an immunochromatographic test, commonly utilized for diagnosis in the region. According to deep amplicon sequencing, *P. vivax* was present in 69.8 percent of the patients, *P. falciparum* in 29.5 percent, and mixed infection in 0.7 percent of the cases. *Plasmodium vivax* was found in 65.6 percent of patients, *P. falciparum* in 27.4 percent, mixed infection in 0.7 percent of patients, and 6.32 percent of positive malaria cases were negative in immunochromatographic but positive in deep amplicon sequencing.

Overall, metabarcoding DNA sequencing improves diagnosis accuracy, resulting in a significant increase in *Plasmodium* infection prevalence estimates. The use of metabarcoding DNA in next-generation sequencing could help in *Plasmodium* infection diagnosis, surveillance, treatment, and control, as well as research into parasite biology.

Keywords: Diagnosis, Surveillance, Illumina, *Plasmodium*, *P. falciparum*, Immunochromatographic, Amplicon sequencing.