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Advancements in Diagnosing and Treatments *Plasmodium knowlesi*: Challenges and Innovations

Moumaris M*

Institute of Medical Sciences, Research and Development Company, France

***Corresponding author:** Mohamed Moumaris, Research and Development Company, 14 avenue René Boylesve 75016 Paris, France, Tel: +33762122825; Email: mohamed.moumaris@sciencesettechnologies.com

Editorial

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Abbreviations

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; LAMP: Loop-mediated Isothermal Amplification; RDTs: Rapid Diagnostic Tests; PCR: Polymerase Chaine Reaction; pfhrp2: *Plasmodium falciparum* Histidine Rich Protein-2; PlasmoDB: Biological Database for the Genus Plasmodium; PTMs: Pig-Tailed Macaques.

Editorial

Diagnosing malaria, especially *Plasmodium knowlesi*, is challenging due to its similarity to other species, which often results in misdiagnoses. While rapid diagnostic tests are unreliable and expensive PCR methods, loop-mediated isothermal amplification (LAMP) presents an option that needs further validation. Effective diagnostics are crucial for accurate treatment. *Plasmodium knowlesi* can be fatal if untreated and is typically managed with artemisinin-based therapies, with no observed drug resistance, highlighting the need for timely diagnosis. *Plasmodium knowlesi* is the main in malaria research in vaccine and drug testing. However, the focus is shifted to pig-tailed macaques, showing consistent replication and immune responses. A new CRISPR/Cas9 technique enhances chromosome segregation in rodent malaria parasites, advancing genetic research with broader applications.

Diagnosing malaria using Giemsa-stained blood films is especially difficult to differentiate *Plasmodium knowlesi* from other species, leading to misdiagnoses. Rapid diagnostic tests (RDTs) for *Plasmodium knowlesi* are generally unreliable. Although PCR methods provide accurate results, they are costly and require specialized equipment. Loop-mediated isothermal amplification (LAMP) offers a simpler alternative but requires further validation [1]. There is a pressing need for improved, affordable, and accessible diagnostic tools to improve diagnostic accuracy, ensure proper treatment, reduce complications, and effectively control malaria. Recent progress in RDT technology has heightened the demand for cost-effective and user-friendly assays in malaria-endemic regions. Recent advancements in malaria RDT research are centered on several key areas, beginning with efforts to increase the sensitivity and specificity of tests for *Plasmodium falciparum* [2]. Researchers are also working to enhance RDTs for *Plasmodium vivax* while developing tests capable of detecting mixte infections. LAMP platforms show potential for detecting *Plasmodium falciparum*, *Plasmodium vivax*, and *Plasmodium knowlesi*. Future efforts should prioritize creating better RDTs for *Plasmodium ovale*, *Plasmodium knowlesi*, and *Plasmodium malariae*, addressing pfhrp2 gene deletions, and maintaining high-performance standards comparable to PCR and expert microscopy [3] (Figure 1).

Plasmodium knowlesi, a swiftly replicating malaria parasite, can be deadly without prompt treatment. The World Health Organization advises using artemisinin-based combination therapy (ACT) or chloroquine for uncomplicated infections. In severe cases, the treatment involves intravenous artesunate followed by ACT. Preliminary studies suggest that combinations like chloroquine with primaquine or artesunate with mefloquine might also be effective. There is currently no evidence of drug resistance, highlighting the necessity of timely diagnosis and treatment to avert severe consequences. Rapid and precise medical intervention is essential for preserving the efficacy of existing therapies [4].

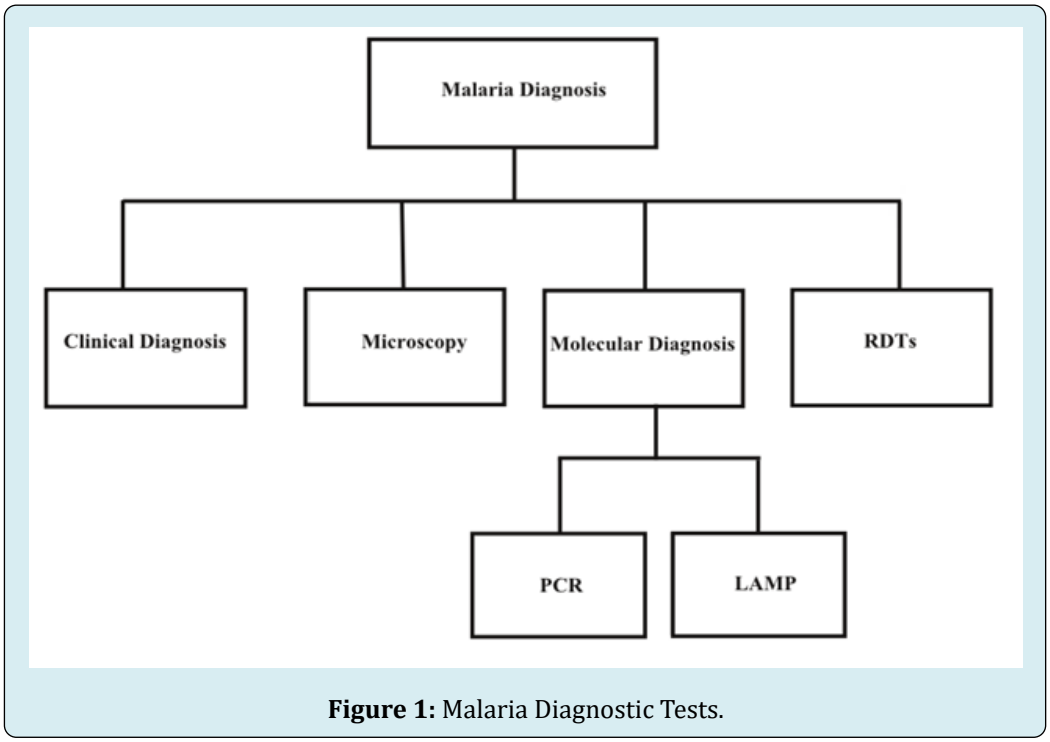

Figure 1: Malaria Diagnostic Tests.

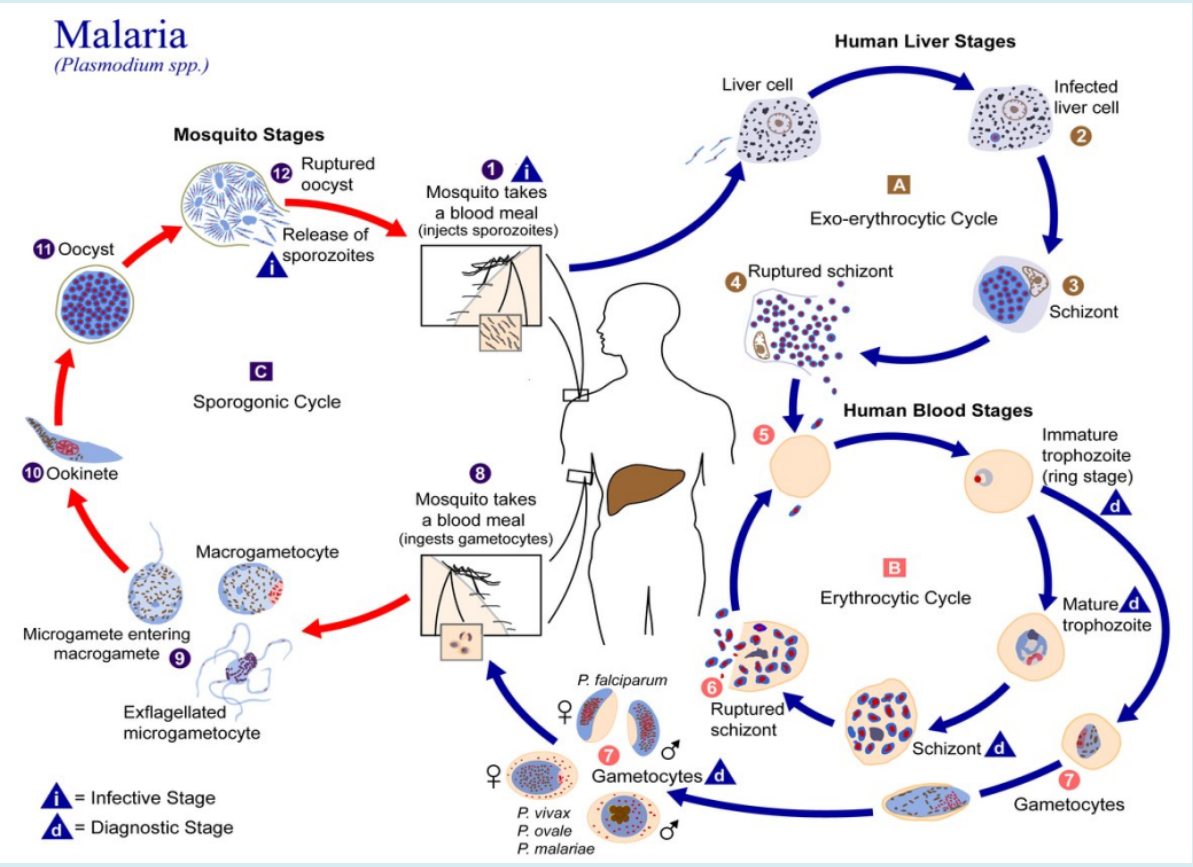

Figure 2 : The life-cycle of Plasmodium parasite. This image is licensed under creative commons attribution.

Plasmodium knowlesi is essential in malaria research due to its ability to infect rhesus macaques, which closely replicates human malaria. This feature allows for repeated studies, supports vaccine development, and is crucial for preclinical drug testing. Initially mistaken for *Plasmodium malariae*, *Plasmodium knowlesi* was overshadowed by *Plasmodium falciparum* until diagnostic advancements revealed its significance as a human pathogen. Maintaining *Plasmodium knowlesi* in continuous culture has revitalized its importance, facilitating in-depth studies on parasite biology, drug resistance, and host-parasite interactions. As a parasite that infects humans and macaques, *Plasmodium knowlesi* offers insights into malaria transmission and its zoonotic potential. Over the past five decades, it has contributed to our understanding of malaria pathogenesis, immunity, and drug development. Advances in diagnostics and culture techniques ensure that *Plasmodium knowlesi* is a focus of malaria research [5,6] (Figure 2).

Plasmodium knowlesi is essential for malaria research, facilitating detailed studies of its lifecycle using rhesus and human red blood cells. The organism genome available on PlasmoDB provides a foundation for investigating gene function, drug resistance, and vaccine development. Additionally, *Plasmodium knowlesi* can be genetically modified and stored for long-term use. Although rhesus macaques have been the traditional hosts for *Plasmodium knowlesi* in vaccine research, they are not the parasite's natural hosts. As natural hosts, pig-tailed macaques (PTMs) demonstrate consistent blood-stage replication, exhibit mild symptoms, and lower parasite densities upon subsequent infections, indicating partial immunity. Gene expression studies during infection have shown active immune responses, establishing PMTs as a reliable and less severe model for studying malaria vaccines. This model can lead to more effective vaccines and better malaria control [7,8].

The CRISPR/Cas9 system integrates telomere and centromere fragments, ensures precise chromosome segregation, and maintains proper telomere function without interfering with development. This innovative approach advances malaria research by modifying chromosome arrangements, offering new insights into gene expression regulation in Plasmodium parasites. Its potential application to other organisms could transform genetic research and the study of chromosome dynamics across various species, underscoring the significance of genome editing in parasitology and molecular biology. This technique holds promise for developing new treatments for parasitic diseases and gaining a deeper understanding of genetic regulation and chromosome structure [9,10].

In conclusion, diagnosing *Plasmodium knowlesi* malaria presents significant challenges, highlighting the urgent need for better and more accessible tools. While advancements in rapid diagnostic tests and loop-mediated isothermal amplification (LAMP) are promising, developing reliable assays for all malaria species is crucial, especially for *Plasmodium knowlesi*. Current treatment options are effective, with no evidence of drug resistance, emphasizing the need for prompt medical interventions. Research on *Plasmodium knowlesi* continues to enhance understanding of malaria, using traditional and innovative models like pig-tailed macaques for vaccine development. Techniques like CRISPR/Cas9 open new avenues for genetic research, providing insights into Plasmodium behavior and resistance. Collaboration among researchers, healthcare providers, and policymakers will enhance diagnostic and treatment strategies to address malaria [11-35].

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