TECHNICAL SERIES

# Pf HRP2 and HRP3 Gene Deletion Mutation In *Plasmodium falciparum* Cases – A Diagnostics Challenge

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# Foreword

Since its Inception in 1988, Tulip Diagnostics (P) Limited, a leading Indian manufacturer of in-vitro diagnostic reagents, kits and instruments has been expanding and growing in its business at a very fast pace. With eleven ISO 13485 certified manufacturing plants pan India and exports to over 88 countries worldwide, the company and its divisions are continuous evolving into important areas of diagnostics including sickle cell anaemia diagnostics tests.

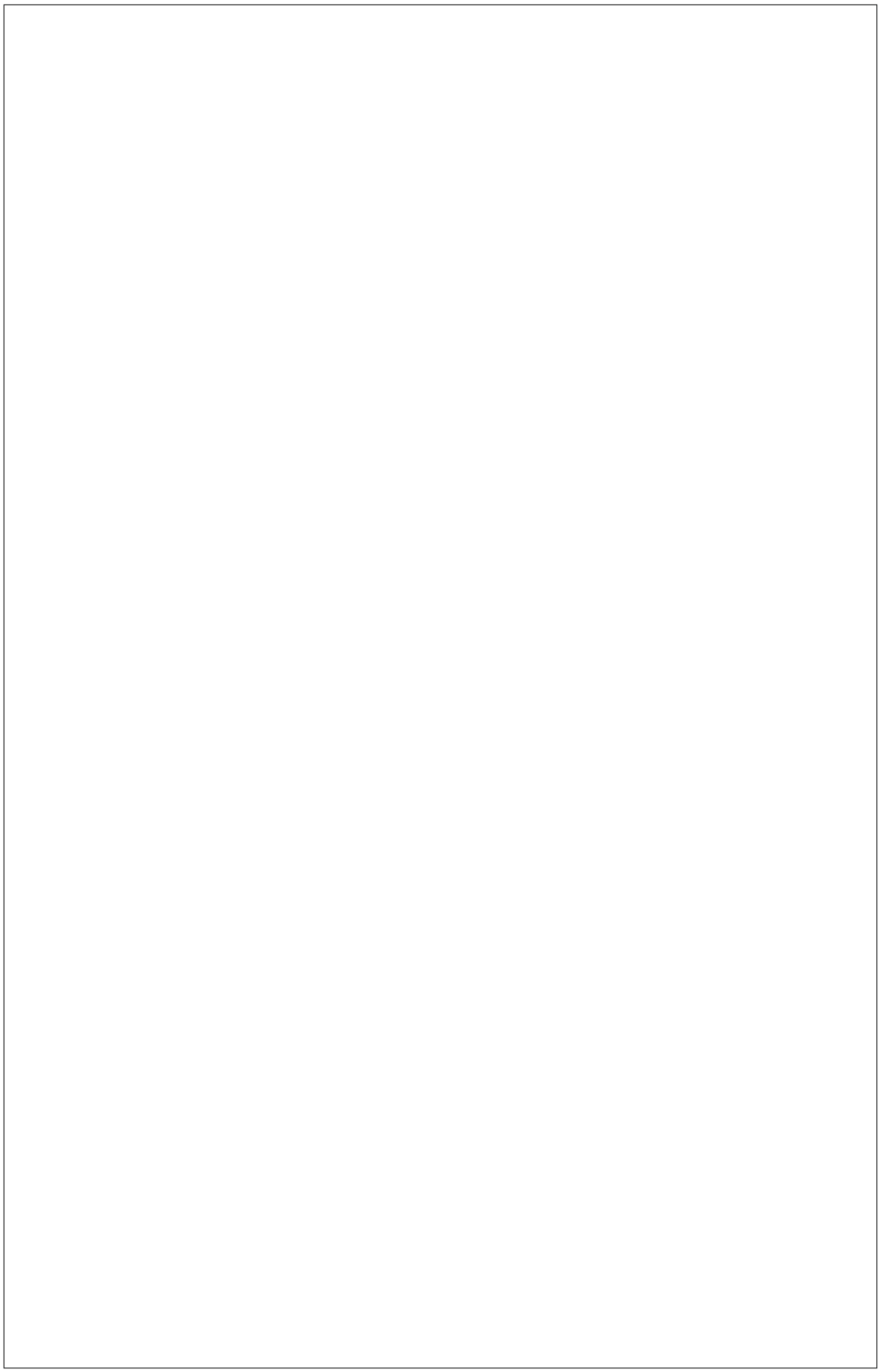
Tulip Diagnostics has always believed that knowledge upgradation is the fundamental basis for better diagnosis and patient care. Publishing of Technical series is one such initiative to make available to the laboratory professionals and clinicians updated knowledge that is vital for them to set trends in their day to day practice.

The occurrence of gene deletion mutations in *Plasmodium falciparum* represents a critical diagnostic challenge and a significant biological threat to global malaria control efforts. As *P. falciparum* has traditionally been identified through the detection of Histidine-Rich Protein 2 (HRP2), which serve as primary diagnostic markers, this mutation of gene deletion creates a serious problem for medical professional to detect Pf infection in malaria prone areas.

In this tech series we have discussed regarding challenge of this mutation of parasite for Pf HRP 2/3 gene deletion hence make difficult to diagnose the disease timely and accurately. We have also discussed the role of RDTs with dual detection capability of Pf pLDH and Pf HRP2 which shall be the appropriate solution to address this gene deletion challenge, especially in resource limited setting where expertise of microscopy or PCR are not available.

It must be noted that the information provided in this tech series is intended to update the knowledge to set advanced trends in malaria diagnostics practice. We hope the contents of this tech series is informative and useful.

Happy Reading!



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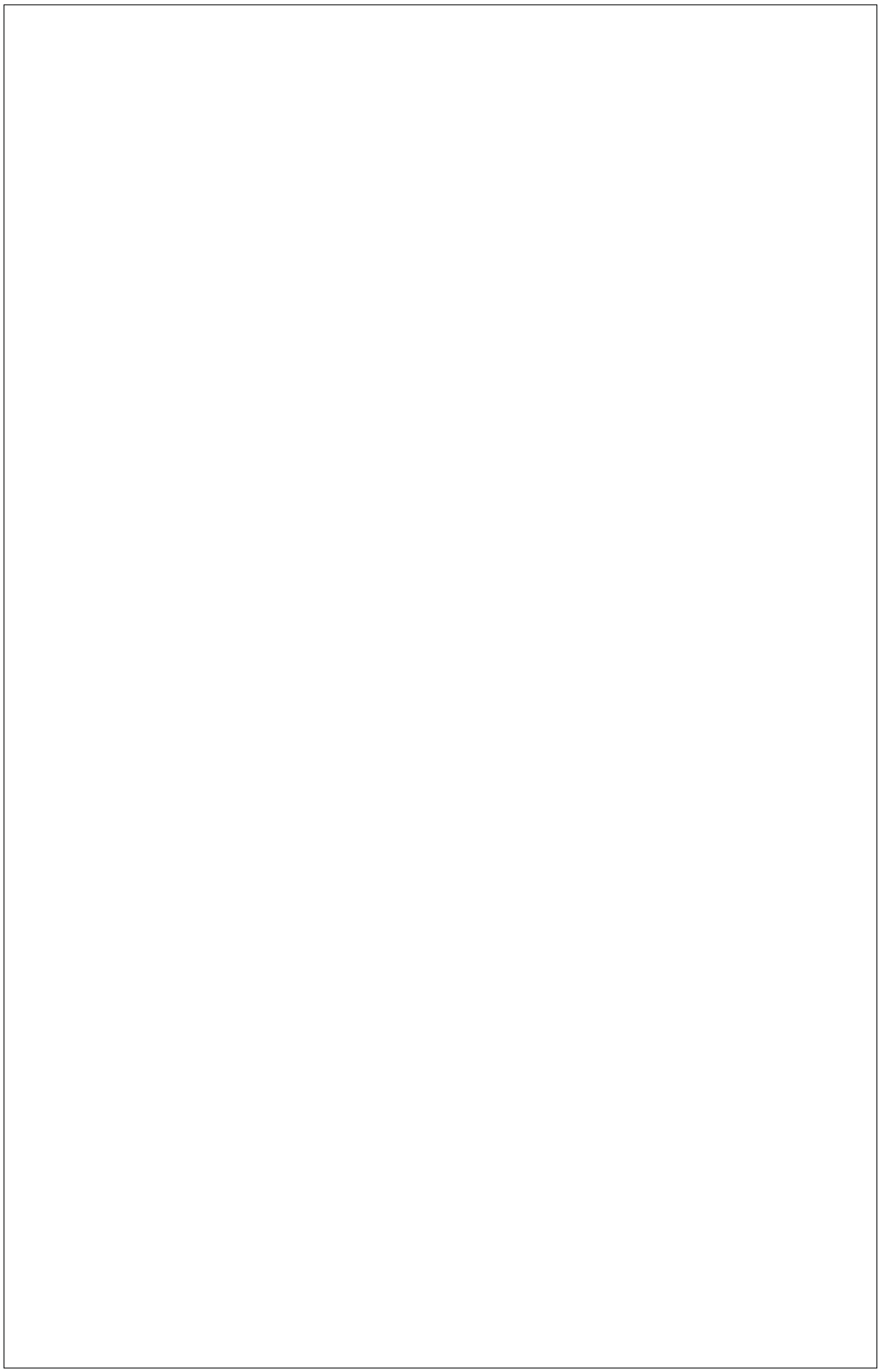


## Overview

The emergence of gene deletion mutations in *Plasmodium falciparum* represents a critical diagnostic challenge and a significant biological threat to global malaria control efforts. As the deadliest species of malaria parasite, *P. falciparum* has traditionally been identified through the detection of Histidine-Rich Protein 2 (HRP2) and HRP3, which serve as primary diagnostic and prognostic markers.

However, the rapid spread of mutant parasites lacking the Pf HRP2 and Pf HRP3 genes has undermined the efficacy of Rapid Diagnostic Tests (RDTs) the most convenient and widely used tools for malaria detection in resource-limited settings. When these genes are deleted, standard RDTs produce false-negative results, leading to untreated infections that can be lethal. The Pf HRP 2 / 3 gene deletion phenomenon (genetic mutation) has been observed across the globe prevalence, ranging from 0.5% to 35%.

Discussion has been attempted to examines the technical and public health implications of this phenomenon, specifically focusing on.

- The Mechanisms of Deletion: How factors like drug pressure, immune selection, and genetic recombination drive these mutations.
- The Global Situation: An overview of how these deletions have spread to 40 out of 47 surveyed countries within just thirteen years.
- Diagnostic Alternatives: A comparison of traditional microscopy, molecular diagnosis (PCR), and the strategic shift toward pLDH-based RDTs to counter the threat.

By understanding the impact of these genetic variations, healthcare systems can better implement the scientific and practical alternative strategies necessary to maintain accurate diagnosis and prevent the further spread of undetectable malaria strains.

## What is Pf HRP 2 and HRP 3 gene deletion mutation in falciparum infection cases?

*Plasmodium falciparum* produces histidine-rich protein 2/3 (*Pf HRP2/3*) that accumulate to high levels in the bloodstream and serve as a diagnostic and prognostic marker for *falciparum* malaria. *P. falciparum* parasites with deletions in the Pf HRP2 gene and paralogue Pf HRP3 gene have now been identified in all malaria-endemic regions, where these parasites do not produce HRP2 and/or HRP3 (a cross-reactive protein). Frequent recombination that occur at the subtelomeric regions contribute to vast genetic variation in the *Pf HRP2* and *Pf HRP3* genes. Recent studies from several African and South American countries showed that extensive variation in the amino acid sequences of Pf HRP2 and Pf HRP3 could influence the frequency of the respective epitopes. Furthermore, deletions of the *Pf HRP* 2/3 gene cause a lack of releasing Pf specific HRP 2/3 during infection of *P. falciparum* malaria. Genetic variation and deletion of Pf HRP2 and Pf HRP3 amino acid sequence cause no release or very low release of Pf HRP 2 protein which is the one of a sensitive detection marker antigen for Malaria detection immunoassays.

Now let us understand diagnosis method of malaria and why Rapid Diagnostics test play a key role in malaria diagnosis. We shall also understand how HRP 2/3 gene deletion become a big challenge in accurate diagnosis of malaria by RDTs.

Ultimately, inaccurate diagnosis threatens ongoing efforts in malaria control and prevention. Therefore, they are often undetectable by HRP2-based RDTs, resulting in false-negative RDT results.

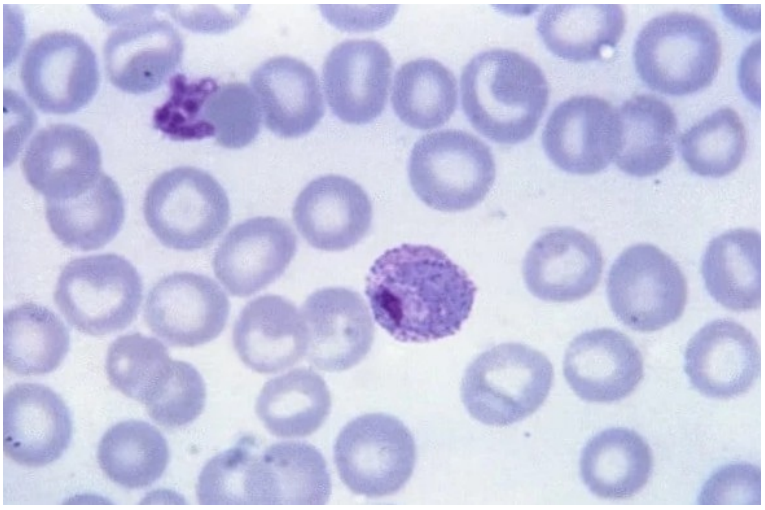
## Different diagnostic method of malaria

**Microscopy (Blood Smear Examination):** This is the "gold standard" for laboratory confirmation of malaria. A blood sample is taken (usually via a finger prick) and spread on a glass slide to make thick and thin blood films. A

laboratory professional examines the stained slides under a microscope to confirm the presence of parasites and determine the species (e.g., *P. falciparum* or *P. vivax*) and quantify the parasite load (parasitaemia).

Parasite levels in the blood can fluctuate, so if the first blood smear is negative but symptoms continue, the test should be repeated every 12–24 hours for a total of three tests to reliably rule out malaria.

Microscopy remains the gold standard for malaria diagnosis because of its high accuracy, especially when performed by skilled laboratory technicians. However, it requires trained staff, electricity, equipment, and time making it challenging in rural or resource-limited settings.



Plasmodium vivax schizont structure

**Rapid diagnostic tests (RDTs)** offer a quicker alternative; they detect parasite antigens in the blood and can give results within 15–20 minutes.

RDTs are particularly useful for point of care testing, faster diagnosis and in remote areas where reliable microscopy is not immediately available.

Malaria rapid diagnostic tests (RDTs) assist in the diagnosis of malaria by qualitative detection of malaria parasites in human blood, particularly where good quality microscopy services cannot be readily provided. Malaria RDTs detect specific antigens (proteins) produced by malaria parasites such as Pf HRP2, pLDH, or Plasmodial aldolase in the blood of infected individuals.

Blood for the test is commonly obtained from a finger-prick.

## Molecular Diagnosis (PCR) for malaria

PCR is most useful for confirming the species of malarial parasite after the diagnosis has been established by either smear microscopy or RDT. Parasite nucleic acids are detected using polymerase chain reaction (PCR). Although this technique may be more sensitive than blood smear microscopy, it is of limited utility for the diagnosis of acutely ill patients in the standard healthcare setting. PCR results are often not available quickly enough to be of value in establishing the diagnosis of malaria infection.

PCR is most useful for confirming the species of malarial parasite after the diagnosis has been established by either smear microscopy or RDT.

## Why rapid test is most convenient for faster malaria detection and treatment?

Malaria Rapid Diagnostic Tests (RDTs) have proven to be an indispensable tool for enhancing efficiency and access to prompt care, especially within large healthcare facilities located in regions heavily burdened by malaria. These kits offer substantial reductions in diagnostic turnaround times because they eliminate the need for complex laboratory processes. In many major hospitals and clinics, the traditional reliance on microscopy is often hampered by an overwhelming combination of high patient loads and inadequate staffing levels, which frequently leaves the laboratory services

completely swamped. This leads to long waiting periods for diagnosis, delaying treatment and potentially worsening patient outcomes. RDTs circumvent this bottleneck by providing a reliable result in minutes at the point of care.

The simplicity of RDTs also allows for vital task shifting within the healthcare system. In many countries, the policy allows *outpatient* nurses or even community health workers to efficiently test suspected malaria patients directly using RDTs even before those patients see a clinician. This decentralization of testing significantly streamlines the patient journey, moving diagnosis out of the centralized laboratory and into the clinical setting where treatment decisions are made. Furthermore, even where traditional microscopes are technically available, the quality and consistency of testing often remain compromised due to a pervasive lack of *adequate* support for essential operational components. This includes shortages of necessary reagents, inconsistent supply chains for basic consumables, frequent failure due to lack of equipment maintenance, and a continuous need for high-quality staff training. In such challenging environments, the simple, self-contained, and standardized nature of RDT kits makes them a vastly superior and more practical choice for ensuring consistent, reliable, and widespread malaria diagnosis compared to maintaining a functioning, high-quality microscopy service.

## Why is gene deletion phenomenon observed in *P. falciparum* cases?

The deletion of the *Plasmodium falciparum* HRP2/3 gene can be triggered by various factors, including:

1. Drug pressure: The use of certain antimalarial drugs, such as artemisinin-based combination therapies (ACTs), can exert selective pressure on the parasite population. This can lead to the emergence of drug-resistant strains, including those with HRP2/3 gene deletions.

2. Immune selection: The host immune system plays a crucial role in controlling malaria infection. The immune response can target specific antigens, including the HRP2 and HRP3 proteins encoded by the HRP2/3 gene. This immune pressure can drive the selection of parasites that have deleted the HRP2/3 gene to evade detection by the host immune system.
3. Genetic recombination: Plasmodium parasites have a high rate of genetic recombination, which can lead to the exchange of genetic material between different parasite strains. This recombination can result in the acquisition of HRP2/3 gene deletions from other parasite strains.
4. Fitness advantage: In some cases, the deletion of the HRP2/3 gene may confer a fitness advantage to the parasite. This could be due to reduced immune recognition or altered parasite biology, allowing the parasite to replicate more efficiently and survive in the host.

As the Pf parasite can randomly delete either of these two genes, random smaller deletions and point mutations can lead to variation of the genomic sequences of Pf HRP2 and Pf HRP3. The variation, especially in Pf HRP2 gene sequence, can hypothetically have an impact on RDT sensitivity, especially at lower parasite densities. To date, Pf HRP2/3 gene variants appear to show geographical clustering similar to other measures of Pf relatedness.

## Why gene deletion matter for falciparum detection?

Malaria, especially the kind caused by the *P. falciparum* parasite, can become a deadly illness very quickly if we don't catch it and treat it fast. Luckily, the invention of Malaria Rapid Diagnostic Tests (RDTs) has been a game-changer, allowing health workers to get results right away and start life-saving treatment in a timely manner.

## The Standard Test Target

Most of these easy-to-use tests are designed to look for a protein called Histidine-Rich Protein 2 (HRP2). This protein is made in huge amounts by the parasite during the stage when it is multiplying in the human bloodstream. The instructions for making this protein come from a gene called Pf HRP2, which sits on chromosome 8 of the parasite's DNA. Because only *P. falciparum* makes HRP2, RDTs based on this protein are excellent at specifically identifying this most dangerous type of malaria. The parasite also has a related gene, Pf HRP3 (found on chromosome 13), which makes a very similar protein called HRP3. Because these two proteins share common features, some HRP2 RDTs can also detect HRP3, offering a bit of built-in redundancy for the test.

## The Deletion episode

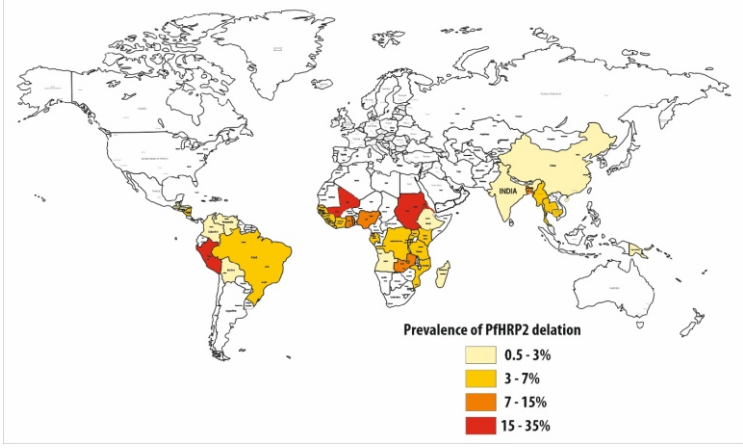
The immense challenge we now face in many malaria-affected regions is the rise of mutant *P. falciparum* parasites where the Pf HRP2 and Pf HRP3 genes have been entirely deleted from the parasite's genetic code. This means the parasite simply cannot produce the HRP2 or HRP3 proteins. When a person is infected with one of these mutant parasites, the most commonly used HRP2-based RDTs fail to find the target protein and mistakenly show a negative result. This false-negative RDT result is lethal, as the patient is sent home untreated while harboring a severe, life-threatening infection. This growing threat of undetectable parasites is a significant biological and public health crisis because it breaks the essential link between rapid diagnosis and timely treatment, undermining decades of malaria control efforts worldwide.

## What is global situation of gene deletion problem?

The global scene regarding the deletion of the Pf HRP2 and Pf HRP3 genes in *P. falciparum* parasites has rapidly transformed from an isolated concern

into a widespread worldwide biological threat to malaria control. The most striking evidence of this escalation is documented by the World Health Organization (WHO), which reported that within just thirteen years of the first *P*f HRP2 -deleted parasite being found in 2010, 40 out of 47 surveyed countries worldwide have now officially confirmed the presence of these Pf HRP2/3 deletions. This high rate of reporting across various continents including high-burden areas in Africa, South America, and Asia underscores that the mutant parasites are virtually everywhere the disease is found. The main danger stems from the high prevalence of these deletions in local parasite populations, as this directly causes a high frequency of false-negative HRP2 RDTs, meaning standard tests fail to detect the infection. This failure to diagnose is lethal for patients and accelerates the spread of the undetectable, resistant strain, effectively undermining the primary diagnostic pillar of global malaria control. Consequently, this widespread biological threat has forced numerous national malaria programs to undertake the complex and resource-intensive process of abandoning their HRP2-based testing and adopting alternative diagnostic strategies to protect their populations from misdiagnosis.

***Global distribution of prevalence of Pf HRP 2 gene deletion cases***



## How can we counter this gene deletion biological challenge in RDT?

The emergence of *P. falciparum* parasites with deletions in the Pf HRP*2* and Pf HRP*3* genes in most malaria-endemic regions poses a significant biological threat to malaria control. These parasites are undetectable by HRP2-based rapid diagnostic tests (RDTs) – the most commonly used test for malaria diagnosis – resulting in false-negative RDT results, which can have lethal consequences for the management of malaria patients.

The primary and most direct countermeasure to the Pf HRP2 and Pf HRP3 gene deletion mutations revolves entirely around a strategic shift in the Rapid Diagnostic Tests (RDTs) used in the field. Since the deletion renders the primary target of over 90% of global RDTs the HRP2 antigen undetectable, public health policy must enforce a switch to RDTs targeting alternative parasite antigens. The World Health Organization (WHO) provides clear guidance that when molecular surveillance confirms that Pf HRP2 deletions are responsible for false-negative RDT results in 5% or more of *P. falciparum* cases in an area, the exclusive use of HRP2-only RDTs must cease immediately to prevent fatal misdiagnosis and curb the spread of the resistant parasite strain.

The recommended alternative RDTs primarily focus on detecting Plasmodium Lactate Dehydrogenase (pLDH), which is an essential metabolic enzyme produced by the parasite and therefore not affected by the deletion of the Pf HRP2/3 genes.

For early diagnosis of falciparum infection, Pf HRP 2 is considered as most sensitive marker compared to the marker like Pf pLDH. Since in falciparum cases early detection and prompt initiation of treatment is crucial to reduce mortality, Pf HRP 2 based has a very critical role and undeniably this marker is an obvious choice for falciparum diagnosis. So, to overcome such gene deletion challenges without compromising the over sensitivity, Combined Detection Pf pLDH along with Pf HRP is become a most appropriate tool to

overcome such biological challenge of Pf HRP 2/3 gene deletion cases without compromising the early sensitive detection capacity.

Combination RDTs that utilize both HRP2 and pLDH analyte is particularly useful, as the presence of a pLDH band with an absent HRP2 band (a Pf-LDH+/HRP2- result) serves as a field-level signal for a potential Pf HRP2-deleted infection, prompting clinicians to exercise extra caution or refer for confirmation.

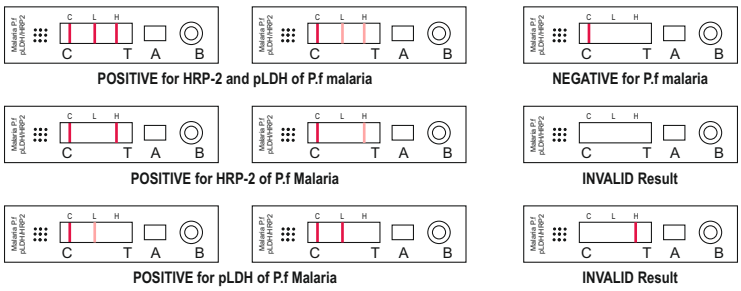
Since Pf HRP 2 antigen is much more sensitive marker than Pf pLDH, Pf HRP 2 based RDT provide more sensitive detection and ensure detection in early stage of infection. Standalone Pf pLDH based RDT has a limitation of low sensitivity for *P. falciparum* detection.

Combination of Pf HRP 2 and Pf pLDH detection overcome the both sensitivity and gene deletion issue and provide most practical solution in filed level malaria diagnosis.

Usually there are two types of combination RDTs available, one is for detection and differentiation of both Pf HRP 2 and Pf pLDH and other one is only detect both Pf HRP 2 and Pf pLDH.

## Result interpretation of both types of Pf detection RDTs

### Standalone Pf detection test result




POSITIVE for HRP-2 and pLDH of P.f malaria

NEGATIVE for P.f malaria

POSITIVE for HRP-2 of P.f Malaria

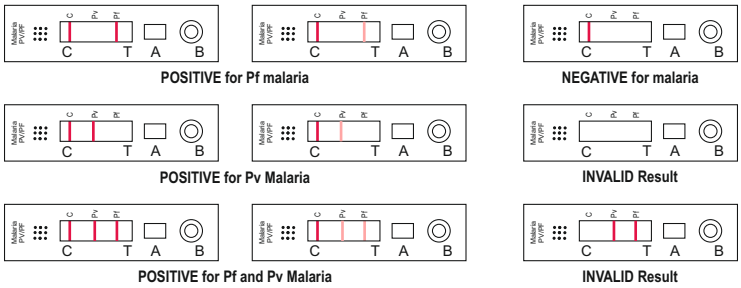
INVALID Result

POSITIVE for pLDH of P.f Malaria

INVALID Result

Note: The L represent the band for Pf pLDH detection while H represent the band Pf HRP2 detection.

### Bivalent Pf/ Pv detection test result




Note: In bivalent tests, usually PF detection band represent for only Pf pLDH detection or combined Pf pLDH and Pf HRP2 detection.

## Conclusion

The *Plasmodium falciparum* parasites lacking histidine-rich protein 2 and 3 (Pf HRP2/3) genes have been reported in several parts of the world. These deletions are known to compromise the effectiveness of HRP2-based malaria rapid diagnostic tests (HRP2-RDT). Recently in a controlled study conducted by an institute of South East Asian country found that 18 out of 19 falciparum samples are Pf HRP2 gene deleted cases. The study has uncovered the criticality of this gene deletion challenge for accurate diagnosis falciparum malaria. In such cases, absence of Pf HRP 2 is big challenge for diagnosis and treatment of falciparum malaria. Pf specific pLDH detection can be a practical option to identify those cases. Since pLDH is much less sensitive marker for falciparum detection test need to be a combined detection system of both the marker i.e. Pf HRP2 and Pf pLDH.

Combination of both diagnostic markers can overcome this biological challenge of HRP2 gene deletion, without compromising the sensitive detection of *P. falciparum* infection.

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