The Biological Factors Affecting the Performance of HRP2-Based Malaria Rapid Diagnostic Tests (RDTs)

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HIGHLIGHT: There are a variety of factors that influence the performance of HRP2-based malaria RDTs. Consideration must be given to these factors to ensure accurate diagnosis of patients when using this tool.

INTRODUCTION

- In 2017, the World Health Organization (WHO) estimated a total of 219 million malaria cases worldwide, a number of cases representing a drop of 18% since 2010 (WHO, 2017).
- Malaria rapid diagnostic tests (RDTs) play a crucial role in the clinical management of malaria cases and disease surveillance due to their affordability and easy use.
- The complex biology of HRP2 poses several limitations to HRP2-based RDTs, but can also be advantageous to estimate parasite sequestration and disease severity.
- This review discusses the numerous biological factors influencing the detection of HRP2 and the consequent implications for malaria clinical management and disease control and elimination, bringing attention to other applications of HRP2 and the need for more effective testing devices.

QUESTION

What biological factors affect the performance of HRP2- based RDTs? Where does HRP2 stand in the fight against malaria?

METHODS

- Google Scholar and Pubmed were utilized to retrieve 60 published articles on the topic of HRP2 between the years 2000-2018.
- Collected papers were used to review factors as well as to create tables on hrp2 gene deletions and repeat sequences.

FUNCTIONALITY OF RDTs

- RDTs detect parasite antigens in blood, mainly the *Plasmodium falciparum* specific histidine-rich protein 2 (HRP2), either alone or in combination with other parasite proteins (WHO, 2015).
- Samples positive for HRP2 will show both control (C) lines and test (T) lines. The intensity of the test band varies depending on the amount of antigen present (WHO, 2015).

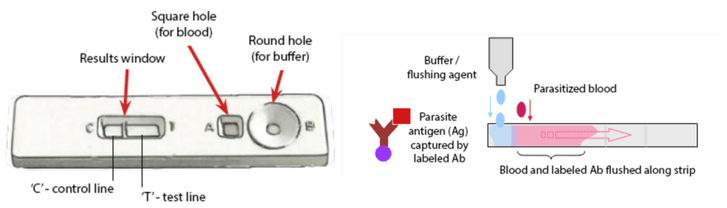

Figure 1: RDT Cassette (left) and mode of action of common RDT format (right) (WHO, 2015)

REVIEW OF FACTORS

Parasitic Factors

- ***Hrp2* and *Hrp3* Polymorphism:** Variation in *hrp2* and *hrp3* due to repeat gene sequences raises concern for the degree of RDT reactivity, although there is currently no established correlation between the number of repeats and the RDT performance in the field. Repeat types 2 and 7 are commonly recognized (Table 1).

Type	Amino acid repeat sequence	HRP2	HRP3	Type	Amino acid repeat sequence	HRP2	HRP3
1	AHHAHHVAD	+	+	18	AHHDD	-	+
2	AHHAHHAAD	+	+	19	AHHAA	+	-
3	AHHAHHAAY	+	-	20	SHHDD	+	+
4	AHH	+	+	21	AHHAHHATY	+	-
5	AHHAHHASD	+	-	22	AHHAHHAGD	+	-
6	AHHATD	+	-	23	ARHAAD	+	-
7	AHHAAD	+	+	24	AHHTHHAAD	+	-
8	AHHAAY	+	-	25	AHHASY	+	-
9	AAY	+	-	26	AHHAHHVSD	+	-
10	AHHAAAHHATD	+	-	27	AHHSHHAAD	+	-
11	AHN	+	-	28	SHHDG	-	+
12	AHHAAAHHEAATH	+	-	29	AHHVAD	-	+
13	AHHASD	+	-	30	AHHAPH	+	-
14	AHHAHHATD	+	-	31	AHHAPD	+	-
15	AHHAHHAAN	-	+	32	AHVDD	+	-
16	AHHAAN	-	+	33	AHHEAA	+	-
17	AHHDG	-	+	34	AHHN	-	+

Table 1: HRP2 Repeat Sequences Table

- ***Hrp2* and *Hrp3* Gene Deletions:** *P. falciparum hrp2* and *hrp3* gene deletions have been identified worldwide, although detection is limited to using PCR, DNA sequencing, and ELISA (Figure 2). Deletions result in false RDT readings, leading to an increase in undiagnosed and untreated malaria cases around the globe. Mathematical modeling can be used to predict future gene deletions (Watson et al., 2017).

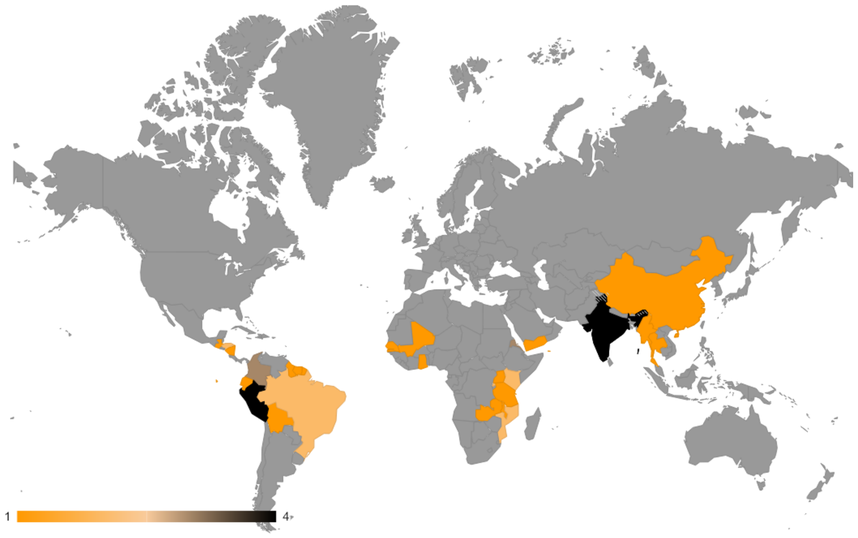

Figure 2: Cross-Continental HRP2 Gene Deletion Comparison. This map was created using Microsoft Excel.

- **HRP2 Persistence:** Persistence of HRP2 in the blood following antimalarial treatment, especially in areas of high transmission, can lead to overmedication and subsequent problems, particularly the emergence of drug resistant or tolerant parasite clones (Mayxay et al., 2001). New highly sensitive RDTs (hsRDTs) show promise in identifying malaria HRP2-positive, asymptomatic patients (Plucinski et al., 2017).
- **HRP2 Sequestration:** *P. falciparum* infected erythrocytes cytoadhere to capillaries located in specific organs of the body. Identifying the concentration of the parasite in infected erythrocytes allows researchers to understand the severity of malaria in the host (Franke-Fayard et al., 2010).

Host Factors

- **Patient Age and Acquired Immunity:** Children are more likely to present a persistent positive RDT reading after the initial test, as they are less likely to have developed an acquired immunity to malaria (Dalrymple et al., 2018).
- **Pregnancy:** RDTs prove to be successful in positively identifying malaria in pregnant mothers as HRP2 aggregates in the placenta and the maternal peripheral blood (Vásquez, 2016). The success of RDTs in malaria screenings of pregnant women are now being used in the new technique of Intermittent Screening and Treatment, or ISTp (Williams et al., 2015).
- **Asymptomatic Patients:** The protozone effect occurs when excess parasite antigens bind with test band antibodies, causing HRP2 to be undetected by RDTs. This phenomenon leads to misdiagnosis of the patient, as their symptoms are often mistaken for a bacterial infection and treated with antibiotics (Williams et al., 2015). Asymptomatic patients with low parasitic density often test negative by RDTs, resulting in failure to receive proper treatment (Orish et al., 2018).

Environmental Factors

- **Moderate to High Transmission Intensities:** RDT performance is shown to be reliable as the parasite densities are greater than 200 parasites/µl and are easily detected (Abba et al., 2014).
- **Low Transmission Intensities:** Low parasitic densities in low endemic countries can lead to both false-positives and false-negatives (Ranadice et al., 2017).

Additional Factors

- **Handler and Operation Error:** Communities with few resources and training programs are more likely to commit handler and operation errors due to inadequate training and medical leadership (Hawkes, 2015).
- **Inadequate Storage:** RDTs in the rural tropics may be exposed to temperatures outside of the recommended range. The high humidity and temperatures above 30 °C can denature the proteins in the RDT causing false results (Guire, 1999).
- **Accessibility of Health Systems:** Rural villages must rely on good logistical planning to transport RDTs, especially in tropical countries, to insure the stability of diagnostic tests (Jorgenson et al., 2006).

CONCLUSION

- There are a variety of factors that affect the performance of HRP2 detection, especially those involving the relationship between parasite and host.
- HRP2 based RDTs remain a valuable tool for diagnosing malaria, although consideration must be given to the numerous influential factors in order to completely diagnose and treat patients.

Future Steps

- Further research must be conducted to determine the extent of both gene repeat sequences and gene deletions worldwide, raising awareness for the need of specialized RDTs.
- Researchers should explore ways to shift RDTs to a more quantitative approach, allowing for the estimation of malaria severity in patients.

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Thank you to Dr. Himanshu Gupta for providing me with mentorship throughout this project and to the Barcelona Institute for Global Health (ISGlobal) for shaping my career trajectory in global health.

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