Innovative technology to combat malaria

Angela Harrison et al describe the burden of a global health problem and how to tackle it

Malaria is a detectable, preventable and curable disease, but the burden on global health is still unacceptably high. Understanding the need for a coordinated effort to combat malaria, the World Health Organization (WHO) initiated the first global effort to eradicate the disease in 1955. Although initially successful, the programme faced numerous setbacks, especially in Africa, and was abandoned in 1972. The integrated fight against malaria was re-ignited at the beginning of the new millennium and substantial gains were made from 2005 in reducing malaria morbidity and mortality (Figure 1). This prompted the WHO to launch the Global Technical Strategy for Malaria 2016–2030, with the ambitious goal of a 90% reduction in the global malaria burden by 2030. However, progress has slowed over the past few years (Figure 1) and the 2020 milestones have not been met. The COVID-19 pandemic and other humanitarian crises have exacerbated the situation and disrupted malaria prevention programmes, as well as diagnosis and treatment of cases, resulting in an estimated increase of 14 million malaria infections and 69,000 deaths in 2020 compared to 2019.

According to the annual WHO World Malaria Report of 2021, there were an estimated 241 million global cases and 627,000 deaths in 2020, with the African region accounting for 95% of the disease burden. The most vulnerable populations are pregnant women and children under the age of five, as the immune system is potentially compromised or still relatively naïve, respectively. The death toll in children under five accounted for 77% of global malaria mortality.

The Plasmodium parasite

Five species of Plasmodium parasites are currently known to cause malaria in humans, but two of them, P. *falciparum* and P. *vivax*, account for most of the disease burden. P. *falciparum* is the most lethal parasite and is responsible for >99% of cases in Africa. P. *vivax* is the predominant parasite in South-East Asia and the Americas.

The life cycle of the malaria parasite involves stages in the mosquito vector, as well as in the liver and red blood cells (RBC) of the human host (Figure 2). The clinical symptoms of malaria are manifested during

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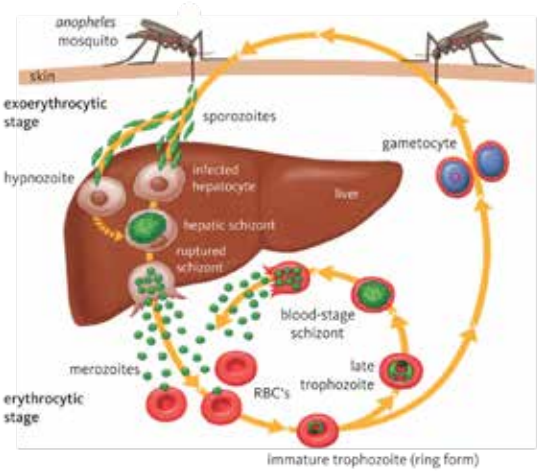
Figure 2. Life cycle of Plasmodium parasites. Source for image: Hill A (2011). 'Vaccines against malaria'. Philosophical Transactions of the Royal Society B.



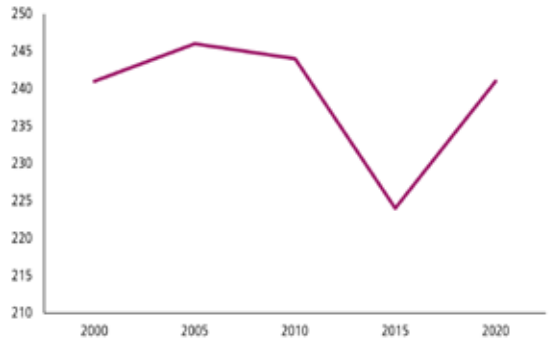
the intraerythrocytic cycle when parasites multiply asexually and new parasites are released when infected RBC lyse, every 48 hours in the case of a P. *falciparum* infection. Some parasites develop into male and female gametocytes, which are the sexual transmissible stages that enable continuation of the parasite lifecycle in the mosquito.

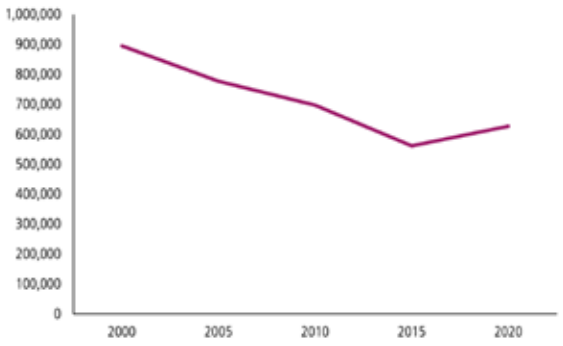
Global strategies to combat malaria

The WHO Global Malaria Programme launched the 'T3: Test. Treat. Track' initiative in 2012 to support malaria endemic countries in achieving universal coverage with testing and treatment and strengthening their surveillance systems. The initiative states that: (1) every suspected case of malaria should be tested, and the diagnosis must be confirmed before treatment commences; (2) every confirmed case must be treated with quality-assured antimalarial medicine depending on the severity of the disease; (3) every confirmed case must be tracked and recorded in a surveillance system to identify populations at risk and assign resources accordingly.

In addition, the WHO Global Technical Strategy for Malaria 2016-2030 (Figure 3), outlines three pillars of an integrated approach to eliminate malaria, with diagnosis and surveillance playing key roles.

Figure 1. Annual estimated malaria cases and deaths from 2000 to 2020 (WHO Malaria report 2021)





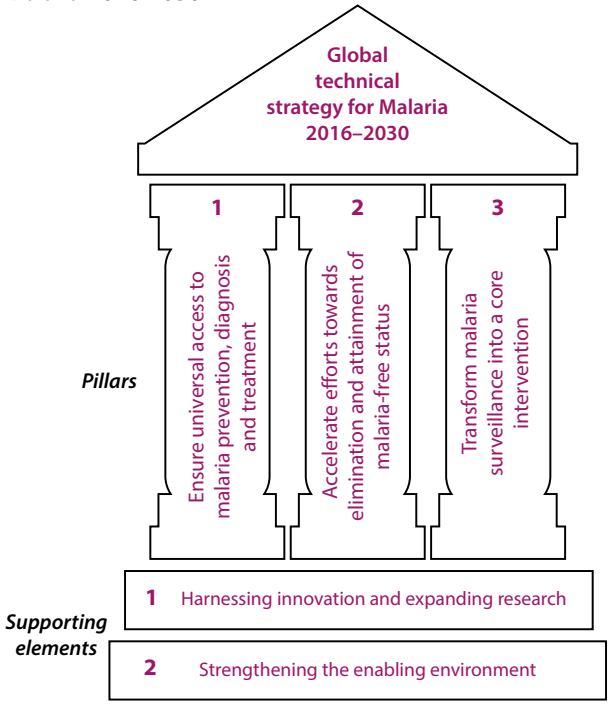
Diagnostic tests for the detection of malaria

The symptoms of malaria are non-specific and clinical suspicion is based primarily on the presence of fever. Therefore, the WHO requires prompt parasitological diagnosis of malaria to confirm the suspected case before treatment commences to ensure rational and appropriate use of antimalarial drugs. The tests recommended by the WHO are microscopy or a rapid diagnostic test (RDT). Apart from these methods, other tests are also used for the detection of an infection, including automated diagnosis (Figure 4 and Table 1). Some of the strengths and weaknesses of current diagnostic tests, especially in the African context, are briefly discussed below.

Microscopy

This is still regarded as the gold standard of malaria diagnostic tests using Giemsa-stained blood smears and oil-immersion light microscopy (Figure 4A). The test has many advantages as outlined in Table 1, however, accurate results depend on the competency and training of the microscopist, quality of the stain and smear, and adequate maintenance of the microscope. In addition, it is time consuming and labour-intensive, and under a high workload it is difficult for a microscopist to maintain accuracy and consistency, especially when the parasitaemia is low.

Figure 3. The WHO Global Technical Strategy for Malaria 2016-2030



Rapid diagnostic test (RDT)

This is a simple and easy immunochromatographic test to detect malaria parasite antigens in a finger-prick blood sample (Figure 4B, Table 1). RDTs can be used in remote rural settings with no electricity and minimal training of personnel is required. RDTs typically detect parasite histidine-rich protein-2 (HRP-2), or in some cases, lactate dehydrogenase (pLDH) or aldolase. Disadvantages include false positive results due to persistence of the HRP-2 antigen after treatment and false negative results due to HRP-2 gene mutations/deletions. The quality of the commercially available kits is variable and lot-to-lot variation is also a concern.

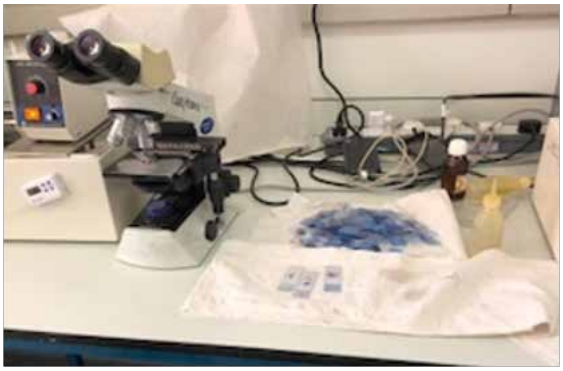
Molecular tests

Nucleic acid-based tests, such as polymerase chain reaction (PCR) and to a lesser extent, loop-mediated isothermal amplification, are used to detect *Plasmodium* genes in blood samples. PCR is highly sensitive and useful at very low parasite densities, and species-specific primers enable speciation. However, highly skilled personnel are required, and specialised equipment and expensive reagents limit the availability of these tests in a routine African setting.

Quantitative buffy coat fluorescence microscopy

QBC is a commercial kit, where the blood sample is stained with a fluorescent dye, acridine orange, and then centrifuged in a capillary tube. The different cell populations in the blood form distinct layers in the capillary and a fluorescence microscope is used to detect malaria parasites in the RBC layer. Similar expertise to conventional microscopy is required, and this labour-intensive method does not allow identification of the *Plasmodium* species.

Figure 4. Diagnostic tests for malaria. Top left: Microscopy. Bottom left: Rapid diagnostic test. Right: Automated detection.



Automated detection of malaria-infected RBC

Several manufacturers of automated haematology analysers have introduced malaria flagging on some of their models, with variable sensitivity and specificity. Algorithms are used to detect the presence of parasitised RBC or haemozoin pigment in white blood cells, which disturb the measurement in certain channels of the analyser.

The Sysmex XN-31 (Figure 4C) is a user-friendly automated haematology analyser where a new laser and innovative reagent have been incorporated, enabling the detection and quantitation of malaria-infected RBC (MI-RBC). The XN-31 reports parasitaemia not only as an absolute number (MI-RBC#) but also as a ratio of the infected RBC to the total RBC (MI-RBC%), and the resulting scattergram provides a visual image of the parasitised RBC clusters (Figure 5). Every measurement generates a concurrent full blood count (FBC), which provides clinicians with important information for clinical correlation, since anaemia is a major contributor to mortality in malaria, and the degree of thrombocytopenia provides an indication of the severity of malaria.

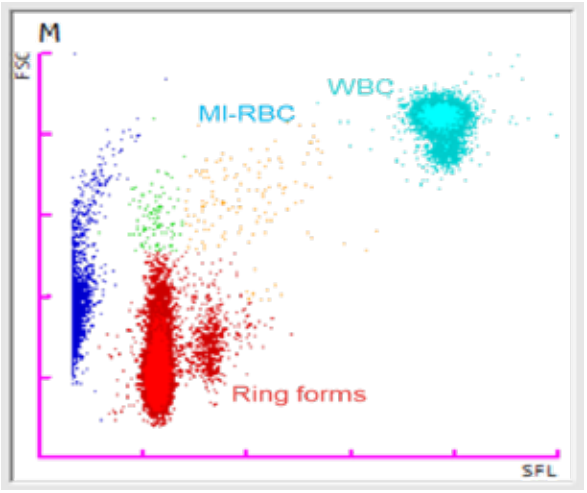
The XN-31 may be used by healthcare professionals in laboratories as an alternative to smear microscopy for rapid and objective diagnosis of malaria in individuals with a clinical suspicion of malaria (Table 1). When patients present with a fever of unknown origin, an FBC is typically requested as part of the diagnostic work-up, and the XN-31 can thus also

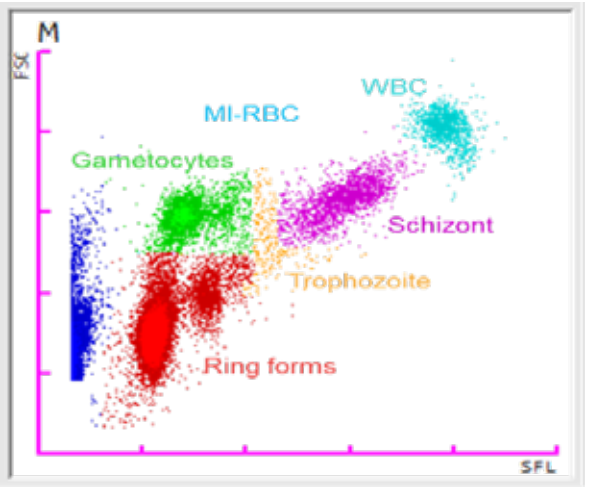
Table 1. Characteristics of diagnostic tests for malaria

	Microscopy	RDT	Sysmex XN-31 analyser
Parasite detection	Direct	Indirect, antigen	Direct
Gametocyte detection	Yes	No	Yes
Species detection	All	Some	All
Subjective	Yes	Yes	No
Quantitative	Yes	No	Yes
Point of Care	No	Yes	No
Automated	No	No	Yes
Time per test	up to 15 minutes	15 minutes	1 minute
LoD parasites/µl	100 - 500 *	100 - 200 **	20
FBC	No	No	Yes
Clinical suspicion	Yes	Yes	No ***

*LoD = Limit of Detection. FBC = Full Blood Count. * An expert microscopist can detect 10 parasites/ul. ** Sensitivity varies depending on the kit manufacturer. *** If the XN-31 is also used for FBC testing*

Figure 5. XN scattergrams from a P. falciparum (left) and a P. vivax (right) blood sample, showing both asexual and sexual life stages of the parasite.





detect unsuspected cases of malaria. This is an important advantage in African countries in the pre-elimination phase; for tourists returning home to non-endemic countries; and for the detection of imported malaria. The analyser provides accurate enumeration and direct detection of the parasites and has excellent sensitivity and specificity as demonstrated in several independent studies conducted in South Africa, Burkina Faso and Colombia.

The XN-31 provides reliable counts even in low parasitaemia (Table 1), which enables clinicians to evaluate the haematological response of the patient during treatment and is independent of operator expertise. This monitoring capability may facilitate the early clinical detection of artemisinin resistance in patients and will also be valuable in malaria drug discovery efforts and clinical trials where monitoring of efficacy is required. The analyser shows excellent suitability in real-life situations within African healthcare centres with high patient numbers, since the automated system generates rapid objective results and minimises human errors.

The role of XN-31 technology

In addition to its role in pillar 1 of the Global Technical Strategy for malaria as an innovative, novel diagnostic tool, the Sysmex XN-31 analyser can also contribute to pillar 2 (elimination) and 3 (surveillance) (Figure 3B).

A key aspect in malaria elimination efforts is to block the lifecycle of the parasite. Semi-immune individuals in Africa serve as a reservoir of sexual gametocytes of P. *falciparum* that are infectious when transmitted to the mosquito and thus ensure continuation of the parasite lifecycle in the vector. The XN-31 can detect gametocytes as a separate cluster and thus identify individuals that should be treated with transmission-blocking drugs to facilitate elimination of malaria.

In terms of malaria surveillance, a recent study at the Malawi Blood Transfusion Services showed the superior utility of the XN-31 in screening blood from asymptomatic donors for malaria parasites compared to microscopy. In addition to improving blood safety and reducing the risk of transfusion transmitted malaria (TTM), the XN-31 results can be used to generate surveillance data. Current surveillance strategies rely mainly on passive case detection and periodic population-based surveys, which are costly and logistically challenging. Continuous data from a readily available asymptomatic blood donor pool could strengthen and complement existing surveillance mechanisms.

Outlook

The global malaria burden has recently escalated and the alarming emergence of partial resistance to artemisinin in some African countries highlights one of the threats hindering malaria elimination efforts. However, the approval of the first malaria RTS,S vaccine in October 2021 is a major advance in protecting vulnerable children living in moderate to high transmission regions from contracting malaria. The use of innovative XN-31 technology as part of an integrated strategy to combat malaria is a promising and positive step in the right direction.

Suggested further reading

1. WHO Global Technical Strategy for Malaria 2016-2030, 2021 update
2. WHO World Malaria Report, 2021
3. WHO Scaling up diagnostics, treatment and surveillance for malaria, 2012
4. WHO Guidelines for malaria, 2021
5. Pillay E, Khodaiji S, Bezuidenhout BC, Litshie M, Coetzer TL. Evaluation of automated malaria diagnosis using the Sysmex XN-30 analyser in a clinical setting. Malar J 2019;18(1),15
6. Post A, Kaboré B, Reuling IJ, Bognini J, van der Heijden W, Diallo S, et al. The XN-30 hematology analyzer for rapid sensitive detection of malaria: a diagnostic accuracy study. BMC Med 2019 May 31;17(1),103
7. Zuluaga Idárraga L, Rios A, Sierra Cifuentes V, Garzón E, Tobón Castaño A, Takehara I, et al. Performance of the hematology analyzer XN 31 prototype in the detection of Plasmodium infections in an endemic region of Colombia. Sci Rep 2021;11,5268
8. M'baya B, Mfune T, Samon A, Hwandih T, Münster M. Evaluation of the Sysmex XN-31 automated analyser for blood donor malaria screening at Malawi Blood Transfusion Services. Vox Sang 2021; doi 10.1111/vox.13208

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