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SEED Haematology

Malaria: the global burden, diagnostic challenges, and new possibilities

Introduction

Malaria is a disease that has plagued humanity since ancient times and still poses a threat for 40 % of the world's population. The disease is caused by a single-celled parasite of the genus *Plasmodium* which is mainly transmitted to humans via the bites of infected female *Anopheles* mosquitoes. Other transmission routes include the passing of the parasite from a mother to the fetus, during a blood transfusion or with the use of contaminated needles. The *Plasmodium* parasite invades liver and red blood cells, where it multiplies and destroys the cells, causing a plethora of symptoms and complications, such as anaemia, fever, headache, chills, and jaundice.

Currently, malaria is focused in tropical and subtropical regions around the equator (Figure 1), where environmental factors favour an all-year round transmission of the disease. A constant warm climate (≥ 20° C) with long rainy seasons provides ideal breeding conditions for the mosquitoes and the multiplication of the parasites inside them. Moreover, the presence of rural areas with poor housing conditions and overcrowding allows the infection to be transmitted more efficiently from individual to individual [1].

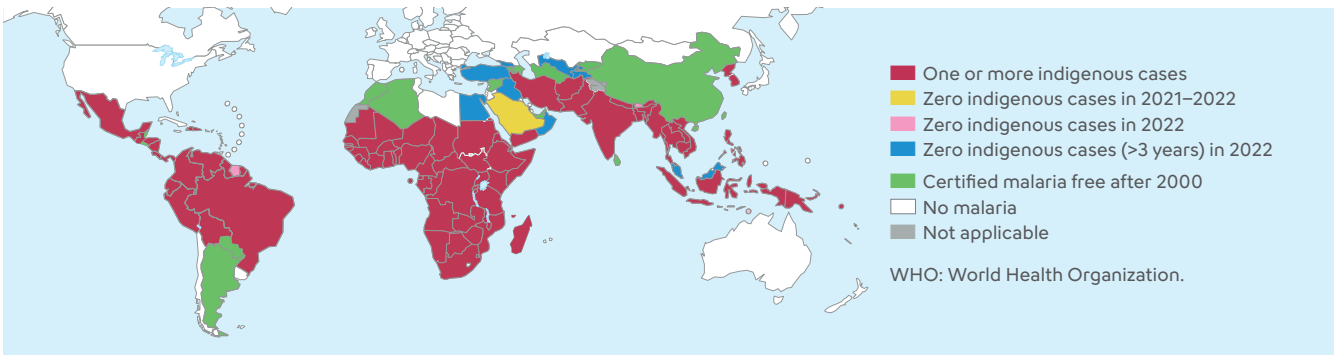

Fig. 1 Countries with indigenous cases and their status in 2022 [1].

* Revision of the original article published in August 2021.

Global health burden

Malaria is a detectable, preventable and curable disease, but the burden on global health is still very high. Understanding the need for a coordinated effort to combat malaria, the World Health Organization (WHO) had already initiated the first global effort to eradicate the disease in 1955. Although initially successful, eliminating malaria from 37 countries, the programme faced numerous setbacks and was abandoned in 1972. After a period of 30 years during which time no organised malaria control programmes were in place resulting in tens of millions of deaths, the fight against malaria was re-ignited in 2000, the results of which can be observed two decades later [2].

According to figures from the WHO, the estimated total annual cases slightly increased from 238 million in 2000 to 249 million in 2022, but with a sharp decrease in the case incidence from 80 to 58.4 per 1000 population at risk, due to the ever-growing human population. Similarly, number of deaths slightly decreased from 736,000 in 2000 to 608,000 in 2022, with the mortality rate dropping from 25 to 14.3 per 100,000 population at risk (Figure 2). Malaria control programmes in that period also lowered the number of malaria endemic countries from 108 to 85, with several more on the verge of acquiring malaria-free status [1].

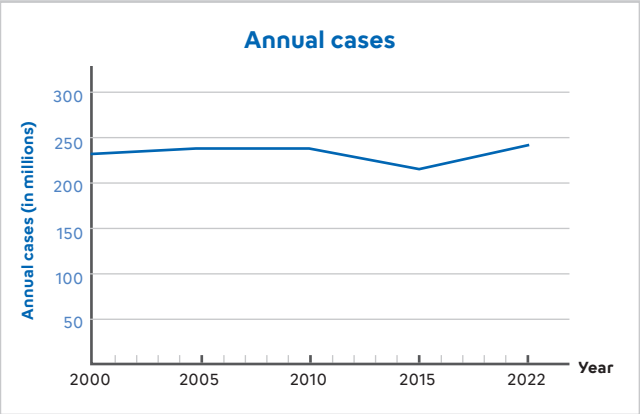

Fig. 2 Annual estimated malaria cases and deaths from 2000 to 2022 [1]. World population data were retrieved from ourworldindata.org.

The largest burden of malaria lies in the African region, which accounts for 94 % of cases and 95% of deaths, and contains 27 out of 29 countries that account for 95 % of malaria cases globally. The most heavily impacted countries are Nigeria and the Democratic Republic of the Congo with 27 % and 12 % of worldwide cases respectively.

The European region has remained malaria free since 2015, but malaria cases are still reported each year, with an incidence of 4,856 cases in 2021. These cases are considered to be imported malaria cases from travellers returning from malaria endemic areas, but effective surveillance, detection, diagnosis and treatment has thus far prevented any malaria resurgence in the region [3].

The most vulnerable populations are pregnant women and children under the age of five. In both groups, the immune system is either potentially compromised or still fairly naive respectively. The burden on the children population is very high, accounting for 76 % of deaths in 2022. An estimated 13 million women were exposed to the malaria parasite during their pregnancy, accounting for 393,000 children with a low birthweight [1].

Over this period of two decades, the WHO Global Malaria Programme launched the 'T3: Test. Treat. Track.' initiative to support the malaria endemic countries in achieving universal coverage with testing and treatment and strengthening their surveillance systems (Figure 3). The initiative states that: (1) every suspected case of malaria should be tested, and the diagnosis must be confirmed with either microscopic examination or a rapid diagnostic test (RDT) before treatment starts; (2) every confirmed case must then be treated with quality-assured antimalarial medicine depending on the severity of the disease; (3) every suspected case, the diagnostic tests conducted, and the treatment administered must be tracked and recorded in a surveillance system in order to identify populations at risk and assign resources accordingly [4].

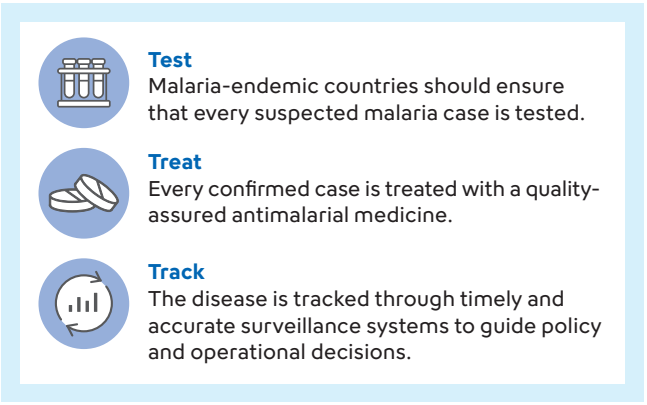

Fig. 3 Summary of T3 strategy by the WHO Global Malaria Programme.

The *Plasmodium* parasite

Five species of the *Plasmodium* parasite are currently known to cause malaria in humans, but two of them, *Plasmodium falciparum* and *Plasmodium vivax,* account for most of the disease burden. *P. falciparum* is the most lethal parasite, accounting for 97.2 % of the cases worldwide and 99.5 % of cases in Africa. *P. vivax* on the other hand has the widest geographical distribution and is the predominant parasite in South-East Asia (45.7 % of cases) and the Americas (72 % of cases) [1].

The transmission and life cycle of the malaria parasite involves stages in the mosquito, and in the liver and red blood cells of the human host (Figures 4 and 5) [5].

a. Exo-erythrocytic cycle; replication in liver
An infection starts with the bite from an infected mosquito that releases sporozoites into the human host. They travel in the bloodstream and infect hepatocytes, where they mature into schizonts and replicate. After a period of time, schizonts are ruptured, destroying the hepatocytes they were residing in, and merozoites are released back into the bloodstream.

b. Erythrocytic cycle; asexual multiplication in red blood cells
Merozoites then infect red blood cells, multiply asexually and develop into immature trophozoites with the distinct ring shape. Some trophozoites further mature to schizonts, and as occurred previously, they rupture, destroying the red blood cells that they infected and releasing merozoites that can infect new red blood cells.

c. Erythrocytic cycle; sexual multiplication in red blood cells
At the stage of immature trophozoites, some cells differentiate into gametocytes, which form the sexual erythrocytic stages; the male microgametocytes and the female macrogametocytes. These cells can be transferred back to mosquitoes during a blood meal.

d. Sporogonic cycle; multiplication in the mosquito
Gametocytes that have been ingested by the mosquito from an infected human enter the stomach, where the microgamete penetrates the macrogamete, generating zygotes that become mobile. The zygotes elongate into ookinetes that invade the midgut wall of the mosquito where they develop oocysts. The oocysts grow in size until they rupture and release sporozoites which make their way into the salivary gland of the mosquito and eventually are transmitted to the next human host with a bite.

Although *P. falciparum* and *P. vivax* follow the same life cycle stages described above, there are several differences between the two parasites along the way. *P. falciparum* spends on average 5.5 days in the first phase inside the liver cells, compared to eight days for *P. vivax,* and releases approximately 30,000 merozoites from each hepatocyte, compared to 10,000 during a *P. vivax* infection. Moreover, schizogony for both species lasts 48 hours, but the incubation period for *P. falciparum* is on average 11 days compared to 14 days for *P. vivax,* and its trophozoites form smaller rings compared to the larger rings of *P. vivax*. Finally, *P. falciparum* can infect red blood cells of all ages, without causing them to enlarge, compared to *P. vivax* that infects younger red blood cells (reticulocytes), causing them to enlarge. This difference in the preference of the target cells can explain the difference in the maximal observed parasitaemia which can be as high as 50 % in severe malaria infection with *P. falciparum*, compared to 2–5 % during a *P. vivax* infection [6].

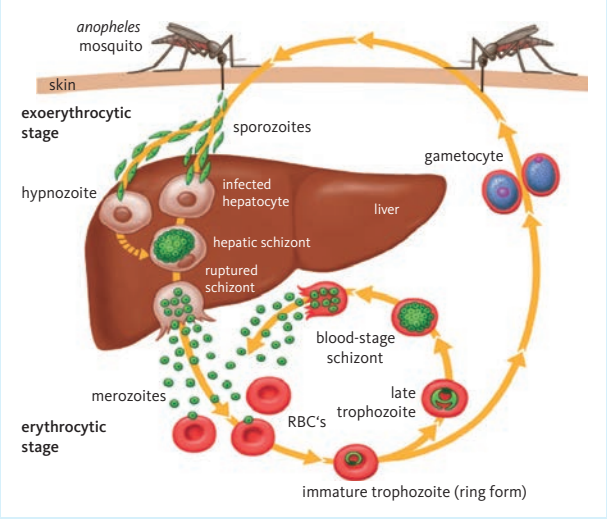

Fig. 4 Life cycle of *Plasmodium* parasite.
Source: Hill A (2011): 'Vaccines against malaria'. Philosophical Transactions of the Royal Society B [5].

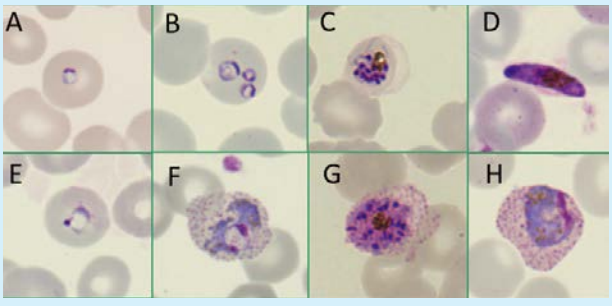

Fig. 5 Morphology of *Plasmodium falciparum* (A–D) and *Plasmodiumvivax* (E–H).
Shown are ring form trophozoites (A, E), mature trophozoites (B, F), schizonts (C, G) and gametocytes (D, H).
Source: Rob Koelewijn and Jaap van Hellemond, Dept. Medical Microbiology & Infectious Diseases, Erasmus MC University Medical Center Rotterdam, The Netherlands.

Clinical manifestation

The clinical manifestation of malaria comes in different forms. Many people have an asymptomatic form of malaria, where they carry the parasite in their blood in very low quantities (parasitaemia less than 5,000 parasites per microlitre of blood), but this comes without signs or symptoms of the disease. A mild anaemia could be observed with less than 0.1% of the red blood cells being parasitised. This form of malaria is mostly found in individuals who have built a strong immunity against the disease through years of constant exposure, such as adolescents and adults from areas of high disease transmission or migrants and visitors from endemic countries.

Mild malaria comes with the manifestation of the classic symptoms, such as fever, chills, headache, and lethargy. The observed parasitaemia is higher than 5,000 parasites per microlitre of blood, also with a form of mild anaemia, where more than 0.1% of the red blood cells are parasitised. Migrants that have developed a semi-immunity usually present with this form of malaria.

The most extreme manifestation of the disease is severe malaria, which is characterised as a complicated disease with signs and symptoms of end organ damage. The parasitaemia level exceeds 100,000 parasites per microlitre of blood, with a manifestation of severe anaemia and up to 10 % of red blood cells parasitised. This type of malaria is mostly found in endemic areas, where children less than five years old, pregnant women and non-exposed travellers have not built immunity yet or have a compromised immune system.

Anaemia

Anaemia can be found in all clinical manifestations of malaria, and moreover, it contributes in one way or another to a significant proportion of deaths. Several mechanisms have been described in order to explain the pathogenesis of malarial anaemia. The most obvious one is the destruction of the parasitised red blood cells themselves when the schizonts are ruptured and the merozoites are released. A greater level of parasitaemia will lead to a higher number of haemolysed cells. At the same time, the spleen has the ability to remove the parasites from within the red blood cells and release them back into the bloodstream. By enlarging, the spleen can facilitate a higher rate of clearance, but those red blood cells now have a reduced life span.

The largest contribution to anaemia, however, comes from the destruction of non-parasitised red blood cells. The membrane of red blood cells, with its characteristic shape and structure, allows them to change their shape while passing through capillaries that are smaller than their diameter and also successfully pass through the filtering in the spleen, a property called deformability. During a malaria infection, the majority of red blood cells lose part of this ability, and with the spleen being stricter in the clearance of abnormal cells, a large number of healthy but slightly rigid cells are removed from the bloodstream and destroyed in the spleen, contributing further to haemolysis and anaemia. Finally, disrupted erythropoiesis has been observed during a malaria infection. Pro-inflammatory cytokines and other factors released during the infection suppress the production of red blood cells and promote the destruction of red precursor cells [7].

Clinical insights and challenges

Early and accurate diagnosis of malaria is vital in the fight against the disease, so that specific treatment can be initiated to prevent complications and reduce mortality. The process of diagnosis is initiated by a suspicion of malaria on the basis of a defined set of clinical criteria, which may vary with the level of malaria endemicity and the types of non-malaria fevers in the area.

The clinical presentation of malaria is one of the least specific of all the major diseases. The causes of fever can range from non-serious viral infections to serious, life-threatening conditions that require immediate, appropriate treatment. Malaria infection can coexist with other life-threatening conditions, such as pneumonia, which also require urgent, appropriate treatment, especially in young children. When managing a febrile patient, it is therefore impossible to know if the condition is due to malaria or another disease solely on the basis of the clinical presentation. For optimal treatment and to save lives, an accurate diagnosis is therefore essential. Confirmatory malaria diagnosis is even more vital in areas with successful malaria control programmes, where the malaria incidence is declining and the likelihood that malaria is the cause of fever is reduced, to make sure that antimalarial drugs are not used inappropriately and that other conditions are recognised and managed properly.

The aim of testing a person presenting with an acute febrile illness for malaria is to confirm the presence or absence of malarial infection and thus decide whether antimalarial drugs are indicated or not.

Current diagnostics tests and new possibilities

1. Rapid diagnostic test (RDT)

A simple and easy immunochromatographic test to detect malaria parasite antigens in a finger-prick blood sample (Figure 6). RDTs do not require water, electricity or laboratory facilities and can be performed in remote rural settings. The performance of RDTs and interpretation of their results require little training, and diagnostic performance does not depend on the availability of trained laboratory technicians. RDTs commonly come in four different formats: cassette, dipstick, card, or hybrid formats which combine different elements. The rapid time to result is approximately 15 minutes.

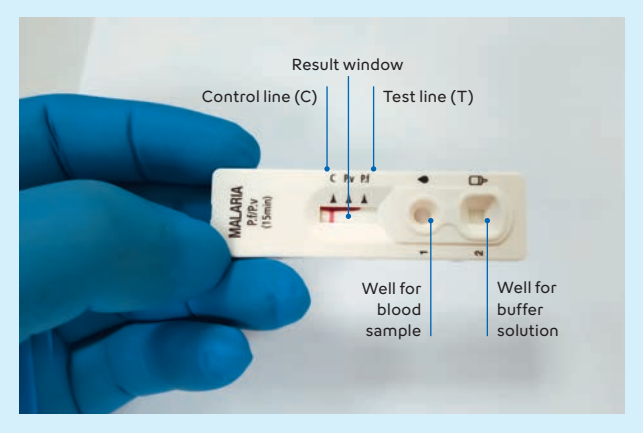

Fig. 6 Schematic representation of an RDT cassette.

Table 1 Challenges in the use of RDTs.

Poor specificity	■ false positives ■ cannot differentiate between recent and new infection
Sensitivity	■ highly variable (~ >100 different RDTs on the market) ■ depends on proper storage conditions ■ depends on species (unless pan-specific, may miss other infections) ■ emerging Histidine-Rich Protein 2 (HRP-2) mutation in *P. falciparum*: false negatives ■ lower limit of detection ~ 100–200 parasites/µL ■ Ultrasensitive HRP-2 based RDTs exist with 10-fold lower limit of detection than conventional RDT (mostly deployed for surveillance)
Diagnostic limitations	■ indirect method (parasite is not directly detected) ■ no quantification ■ cannot be used for monitoring

[1] Thin smears consist of blood spread in a layer such that the thickness decreases progressively towards the feathered edge. On the feathered edge, the cells should be in a monolayer, not touching one another. The thin film is used to confirm the malaria parasite species.

[2] The thick smear consists of many layers of red and white blood cells. During staining, the haemoglobin in the red cells dissolves (dehaemoglobinisation), so that large amounts of blood can be examined quickly and easily. The blood components (including parasites, if any) are more concentrated (approximately 30×) than in an equal area of a thin smear. Thick smears allow a more efficient detection of parasites (increased sensitivity). However, they do not permit an optimal review of parasite morphology and they are often not adequate for species identification of malaria parasites.

2. Microscopy

The WHO recommends prompt parasite-based diagnosis by microscopy or malaria RDT in all patients with suspected malaria before antimalarial treatment is administered [8]. Giemsa is the classical stain used for malaria microscopy, and diagnosis requires examination of both thin[1] and thick[2] films from the same patient.

However, accurate microscopy results depend on the availability of a competent microscopist using good-quality staining reagents for examination and well-prepared slides under a well-maintained microscope with an adequate light source. Aside from this, a low-to-moderate workload should not be exceeded in order to avoid fatigue. It has therefore been difficult to maintain good-quality microscopy, especially in peripheral health services, where most patients seek treatment in malaria endemic countries. The private sector, which also provides laboratory services to a large part of the population in some countries, often remains severely under-resourced.

The dilemma for non-endemic countries is that all the technical requirements for smear preparation are provided, but malaria is so infrequently seen in routine haematology laboratories such that most microscopists lack experience and cases might not be recognised.

Currently, malaria is diagnosed using two main methods: RDT and microscopy. According to the WHO and the Centers for Disease Control and Prevention (CDC), both solutions have critical faults in effective diagnosis of malaria. RDT is affordable, fast and easy, but can only be used for screening. Microscopy is a gold standard method for diagnosing malaria, but relies heavily on a proper laboratory set-up and the expertise of the personnel.

Table 2 Challenges of microscopy related to malaria diagnostics.

Results are variable	■ quality of smear ■ experience of microscopist ■ time spent scanning each smear, i.e. high-power fields (HPF) examined before declaring a smear negative
Microscopy is still the gold standard but has a 'false negative' rate	■ sensitivity not always good enough especially in pregnancy ■ sensitivity influenced by workload ■ routine LoD ~ 100–500 parasites/µL ■ expert LoD ~ 5–50 parasites/µL
Diagnostic limitations	■ subjective result – dependent on the experience of microscopist ■ limited availability of the experienced staff ■ not standardised ■ manual external quality assurance programmes (per country)

3. Molecular tests

Polymerase chain reaction (PCR) is a popular molecular *method to detect Plasmodium genome in blood samples.* The principle of PCR reaction was developed to multiply (amplify) specific pieces of DNA. In the field of diagnostics, the specific amplification enables the detection of e.g. microorganisms in human specimen. It is generally considered a rapid (relative to culture) and highly specific method for the diagnosis of infectious diseases. PCR is an attractive addition to microscopy for confirmatory identification of *Plasmodium* parasites in clinical specimens. There are subtypes of PCR reaction for different purposes e.g. classic PCR for the qualitative confirmation whereas quantitative PCR (qPCR) can assess the amount of genome – i.e. parasites – in a sample.

Identification of all species (giving a positive result for the detection of malaria) and differentiation between species with the identification of the specific species present, is dependent on the choice of the PCR primers (short DNA pieces which are used to target a specific DNA sequence e.g. those in the different malaria parasites) and assay design. PCR is largely used as a confirmatory test, notably for speciation, and hence the testing set-up is largely manual with reagents developed in house, although there are some commercial malaria PCR reagent kits available.

Another emerging molecular method is LAMP, the loop-mediated isothermal amplification. Similar to PCR a specific DNA sequence is targeted by primer molecules and amplified which enables the detection. LAMP differs from PCR as the amplification is carried out at constant temperature ('isothermal') by using a different type of polymerase enzyme. Due to this simplicity together with a certain robustness towards interferences LAMP holds the potential for a simple but specific screening test for infectious diseases in the field.

Some automated molecular malaria screening platforms have been developed, largely based on LAMP technology and are in use for screening and/or confirmation of a malaria diagnosis in some facilities, predominantly in non-endemic countries.

The challenges and limitations for the automated platforms are highly dependent on the individual commercial product and thus not shown in the table here.

Given the fact that both, PCR as well as LAMP, refer to the genome of the parasite, none of them are suitable for detecting specific life cycle stages of the parasite. This would require a specialised analysis by reverse transcriptase PCR (RT-PCR) on transcriptome level.

Table 3 Challenges and limitations of the molecular tests PCR and LAMP.

	PCR	LAMP
Availability	■ limited availability – only in specialised centres because of specialised technical equipment ■ high costs compared to other routine tests ■ not available on demand (batched ~ 6 hours) ■ high level of expertise required	■ better availability due to less specialised equipment ■ less expensive than PCR due to less specialised equipment ■ available on demand, no high throughput method ■ less expertise required than PCR
Sensitivity	■ very high sensitivity, but also high variability depending on: • target gene • number of targets • age/quality of sample • DNA/RNA quality ■ technology used (e.g. qualitative vs quantitative)	■ very high sensitivity, but also high variability depending on: • target gene • number of targets • age/quality of sample • DNA/RNA quality ■ technology used (e.g. qualitative vs quantitative)
Diagnostic limitations	■ no standardisation between laboratories, thus results may vary, depending on chosen technology, kit, target gene etc. ■ highly complex identification of parasite life stages, notably gametocytes (requires a dedicated assay (RT-PCR)) ■ PCR amplicon contamination of the work area poses a serious risk for false positive results	■ commercial kits allow standardisation ■ identification of parasite life stages not possible ■ PCR amplicon contamination of the work area poses a serious risk for false positive results

4. Haematology analyser for quantifying malaria-infected red blood cells

Traditionally, several manufacturers of haematology analysers have introduced malaria flagging on different models of analysers. The sensitivity and specificity of those flags varies a lot and therefore can never become or replace a screening method. The general idea is that manufacturers use algorithms to detect the possible presence of parasitised red blood cells that disturb the measurement in certain channels.

The XN-31 is an automated haematology analyser where a new laser has been incorporated and, in combination with a new reagent, the detection and counting of malaria-infected red blood cells (MI-RBC) is enabled. 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 red blood cells clusters. Every measurement generates a concurrent complete blood count (CBC), 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 [7].

The XN-31 is intended to be used by healthcare professional and any properly trained laboratory personnel for the rapid and objective identification of parasite-infected red blood cells. Operation is possible 24/7 and is not tied to any requirements for expertise in malaria diagnostics. The analyser provides accurate enumeration and direct detection of the parasite-infected red blood cells with a demonstrated sensitivity of 100 % (95 % CI: 97.0100 %) and a specificity of 100 % (95 % CI: 92.6100 %) [9].

Finally, it can support the mass testing and the quick and easy measurement of parasite clearance, with a standardised method that gives consistent objective results independent of expertise. The XN-31 has a consistent standard limit of quantification (LoQ) of 20 parasites/µL, providing reliable counts even in low parasitaemia, which enable clinicians to evaluate the haematological response of the patient during treatment and is independent of operator expertise. The enumeration is also not affected by parasite mutations, such as the histidine-rich protein 2 *(Hrp2)* mutation of *P. falciparum* which impacts the RDT performance.

Outlook

Globally, there has been progress in decreasing malaria burden and mortality due to the expanded coverage of effective interventions and increased resources.

The future of malaria control is critically impacted by external factors including population growth, migration, poverty, inequity, complex emergencies and climate change, combined with weak health systems and biological threats, such as insecticide and drug resistance.

The Global Technical Strategy for Malaria aims to [10]:

- ensure universal access to malaria prevention, diagnosis and treatment.
- accelerate efforts towards elimination and attainment of malaria-free status.
- transform malaria surveillance into a core intervention.

The diagnostic tools which are available 24/7 and are not linked to the skills of the staff could support the Global Technical Strategy and should be used in a way that expands the access to quality early diagnosis and treatment.

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