In the current issue of Clinical Chemistry, Cheng et al. describe their application of a recently developed commercial kit to perform molecular diagnosis of malaria by a capture and ligation probe-PCR (CLIP-PCR) strategy (1). Some review of the efforts directed against malaria is necessary to understand how CLIP-PCR will integrate into the current global malaria elimination effort.

Malaria Control, Elimination, and Eradication

Sixty years ago, the WHO launched the Global Malaria Eradication Program (GMEP) (1955–1969). The GMEP was successful in eliminating malaria from numerous regions in temperate climates (including parts of southern Europe, the United States, the former Soviet Union, the Middle East, Southeast Asia, India, Sri Lanka, Mexico, Venezuela, and the Caribbean Islands) (2). Operationally, the GMEP was largely equipped with insecticides—dichloro-diphenyl-trichloroethane (DDT) with residual longevity was central to the mission (3)—and motivated by evidence for interruption of malaria transmission from a control campaign in Greece and additional success in interrupting malaria and yellow fever transmission during construction of the Panama Canal (3). Unfortunately, numerous biological (mosquito insecticide resistance and parasite drug resistance), socioeconomic, and political factors conspired against the GMEP, and the global effort to eradicate malaria fell well short of its goals (2, 3). Additionally, resurgence of disease has been well documented in numerous countries where malaria transmission had reached or was approaching elimination (4). Thus, whereas some campaigns against malaria have been durable and long-lived, others were very fragile.

The effort to eliminate malaria was rejuvenated beginning with the 1997 International Conference on Malaria in Africa: Challenges and Opportunities Cooperation, held in Dakar, Senegal (5). At that time, malaria was killing an estimated 1.5–2.7 million people annually (3–5 people per min), insecticide resistance was being observed in many of the Anopheles species vectors (2, 6), and parasite resistance to chloroquine had swept across Africa. Resistance to chloroquine had been demonstrated as directly linked to substantial increases in malaria mortality throughout Africa, and malaria experts around the world were signaling a “malaria disaster” there (7). This threat prompted participation in the Dakar meeting from leadership of the NIH, the UK Wellcome Trust, Institut Pasteur (Paris), WHO Tropical Disease Research Program, and the World Bank (5). Numerous malaria elimination initiatives can track their origins to this meeting, including the Multilateral Initiative on Malaria and ultimately the Global Fund to Fight AIDS, Tuberculosis, and Malaria (8, 9).

By 1997, substantial changes had occurred to increase technological capacities for malaria laboratory and epidemiological studies. It had become possible to culture Plasmodium falciparum, among the most important human malaria parasites (10). Recombinant DNA technology made it possible to clone genes and express proteins that could be used to develop diagnostic assays (11) and vaccines (12). Furthermore, the PCR had revolutionized the ability to detect sequences specific to each of the human malaria parasite species, and with the development of highly sensitive molecular diagnostic assays, researchers were poised to transform perspectives regarding malaria epidemiology (13).

The current global effort against malaria is focused by regional goals to turn back transmission of this disease and a recognition that it will be important to couple control and elimination efforts with research and a strong appreciation of the heterogeneous characteristics of the disease in different climates and ecological settings. In the past 5 years, the Malaria Eradication Research Agenda (malERA) has published several articles reviewing successes and failures of the GMEP and reminding the community about definitions (Fig. 1), strategies, and tools to eradicate malaria (14). As malERA has stated, there must be a “[s]ustained commitment from local communities, civil society, policy leaders, and the scientific community, together with a massive effort to build a strong base of researchers from the endemic areas” for this new agenda to succeed (14). Primary components of the malaria elimination toolkit include existing long-lasting...
insecticide-treated bed nets (LLINs) to protect against exposure to mosquito transmission of malaria parasites, fast-acting artemisinin-based drugs formulated in combination with other antimalarial drugs [artemisinin-based combination therapy (ACT)], and rapid diagnostic tests (RDTs) capable of detecting malaria parasite proteins in the blood of infected people.

Comparison of Malaria Diagnostic Methods

First-level comparisons of malaria diagnostic methods (microscopy, RDTs, and nucleic acid–based tests) have been discussed in the literature for years (15). Generally, these comparisons agree that microscopy and RDTs are comparable in sensitivity (limits of detection [LODs] approximately 40–50 parasitized cells per microliter of blood). In contrast, microscopy has the capability to offer species- and developmental stage–specific identification of each human malaria parasite that RDTs are not able to deliver. Many PCR-based diagnostic assays have been developed, and their comparative LODs in detecting malaria infections are approximately 2 orders of magnitude lower than that of microscopy and RDT (0.5 parasitized cells per microliter of blood). An added benefit of PCR diagnosis is the superior capacity for identifying all human malaria parasite species. The PCR surrenders the ability to evaluate important morphological features of infected red cells to microscopy. Both microscopy and PCR are incapable of delivering point-of-care diagnosis possible by RDTs in remote rural settings where even healthcare facilities are understaffed and underresourced.

With powerful PCR-based diagnostic assays, a wide range of clinical and field-based studies have been published to advance perspectives on the complexity of malaria epidemiology because of the ability to detect the reservoir of parasites that are below the LOD of microscopy and RDT (16). Malaria PCR diagnostic assays have incorporated numerous strategies to differentiate among the different human malaria species through genus-specific PCR amplification targeting the 18S ribosomal RNA (rRNA) gene sequences that include regions common throughout the Plasmodium genus (which facilitates use of unbiased PCR primers to amplify all human parasite species) surrounding regions that are species-specific (which enables use of post-PCR methods to distinguish species) (17). These assays have targeted both 18S RNA (18) to boost sensitivity of detecting expressed gene sequence (thousands of copies compared to <10 copies of Plasmodium rRNA genes) or DNA sequence (13) to improve the durability of Plasmodium nucleic acid. Additionally, loop-mediated isothermal amplification shows potential to enable highly sensitive, nucleic acid–based malaria diagnosis in remote health care settings (19) to address a key limitation of nucleic acid–based tests.

Despite the superior sensitivity and specificity of nucleic acid–based tests, stakeholders (UNICEF, President’s Malaria Initiative, Global Fund) have chosen RDTs as the approach for malaria diagnosis to monitor the impact of LLINs and ACT and measure progress toward the global health goal of eliminating malaria as a public health threat for a predicted population at risk of approximately 2.5 billion people. Reasoning that favors

Operational Goals

Control – Reducing the malaria incidence, prevalence, morbidity, or mortality to a locally acceptable level resulting from deliberate efforts; continued intervention measures would be required to maintain control status.

Elimination – Reducing to zero the incidence of locally transmitted infection in a defined geographical area resulting from deliberate efforts; continued intervention measures would be required to prevent reestablishment of transmission.

Eradication – Permanent reduction to zero of the global incidence of malaria as a result of deliberate efforts; intervention efforts would no longer be needed.

Recognition that 5 species now cause human malaria – Eradication of P. falciparum would constitute a historic public health achievement. However as P. falciparum and P. vivax are equally prevalent (often coendemic) and both contribute to the global public health burden of malaria, malERA recognized the importance of eradicating both species. Additionally, P. malariae, P. ovale and P. knowlesi are less prevalent and regionally transmitted human malaria parasites.

Fig. 1. Operational goals for malaria eradication as defined by Alonso et al. (14).
the preference of RDTs is the convenience inherent in this platform (no electricity, minimal training to enable test performance by community volunteers, minimal sample handling, and rapid diagnosis and point-of-care treatment delivery).

**Strengths and Limitations of CLIP-PCR**

Consistent with other nucleic acid–based tests that target 18S rRNA, CLIP-PCR applied by Cheng et al. may benefit from the parasites amplifying the target sequence themselves (1). Additionally, there is possibility that the methods used by Cheng et al. to capture 18S rRNA, which are reported to avoid DNA extraction, may be highly efficient and contribute to the reported superior evaluation of pooled dried blood spot samples. Specifically, in their study, Cheng et al. performed tests on serial dilutions of an in vitro culture of *P. falciparum* (laboratory-adapted strain 3D7) showing that their LOD was 0.01 parasitized cells per microliter of blood (1). Furthermore, they reported not seeing any reduced capacity to detect their *P. falciparum* target sequence even in pools of up to 26 samples that might dilute nucleic acid concentration when combined with uninfected samples. Whereas those authors have reduced costs of their malaria diagnosis significantly by reducing the number of assays to <500 from 3358 samples, Hsiang et al. (targeting the multiple copy mitochondrial DNA, *P. falciparum* cytochrome b gene) have also been successful in performing malaria diagnosis by pooled screening of 20–25 dried blood spot samples (20).

An important limitation of the study is one encountered by all of the other nucleic acid–based studies that have been developed. Although these studies exhibit superior sensitivity in detecting malaria parasites, they are laboratory-based. This presents the dilemma that confronts malaria elimination stakeholders. Should sensitivity for *Plasmodium* species detection be forfeited for the ease of RTD performance? This question will be debated for many more years, and time will tell whether malaria is able to hide in a reservoir that is below the RDT LOD.

A further limitation of CLIP-PCR is its overall lack of transparency. The methods provided by Cheng et al. direct readers and potentially interested practitioners to the commercial source of all CLIP-PCR assay materials, Diacurate (www.diacurate.com). Between the article and the information available on the company website, there is no information stating the sequences of the capture or detection probes or the components (concentrations) of the assay lysis mixture, wash buffers, or ligation mix. In keeping with the competitive and open spirit of the malaria research community, further details are needed that would enable others to evaluate the strengths and limitations of CLIP-PCR.

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