Malaria and the ‘last’ parasite: how can technology help?

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Abstract
Malaria, together with HIV/AIDS, tuberculosis and hepatitis are the four most deadly infectious diseases globally. Progress in eliminating malaria has saved millions of lives, but also creates new challenges in detecting the ‘last parasite’. Effective and accurate detection of malaria infections, both in symptomatic and asymptomatic individuals are needed. In this review, the current progress in developing new diagnostic tools to fight malaria is presented. An ideal rapid test for malaria elimination is envisioned with examples to demonstrate how innovative technologies can assist the global defeat against this disease. Diagnostic gaps where technology can bring an impact to the elimination campaign for malaria are identified. Finally, how a combination of microfluidic-based technologies and smartphone-based read-outs could potentially represent the next generation of rapid diagnostic tests is discussed.

Keywords: Malaria, Rapid diagnostic tests, Elimination, Microfluidics, Smartphones

The burden of malaria
The first record of malaria fevers dates back to the 5th century BC [1]. Today, malaria remains one of the four most life-threatening infectious diseases worldwide, together with tuberculosis, HIV/AIDS and hepatitis [2]. Latest data published by the World Health Organization (WHO) are staggering: more than 216 million cases in 91 countries and more than 400,000 deaths occurred globally in 2016 [3]. These figures are the same as in 2015, indicating that despite the unprecedented efforts in recent years, progress has stalled. This calls for more effective tools to reduce malaria and finally to eliminate this scourge. If this historical milestone can be accomplished, it could save the global economies $2 trillion by 2040 [4].

Current diagnostic technologies and the challenges of detecting the ‘last’ parasite
This review only focuses on relevant innovative diagnostic technologies for malaria elimination settings where the malaria transmission is low; therefore, there is a critical need to detect asymptomatic individuals. Together with other effective interventions, ultra-sensitive rapid diagnostic tests are much needed to identify the invisible reservoirs. The role of innovative tools becomes crucial in the fight against malaria and the WHO identifies three strategic pillars (universal access to prevention, drugs and diagnosis, elimination and surveillance), of which accurate and effective diagnostics at the point-of-care (POC) is the first step towards appropriate diagnosis and treatment for malaria infection [5, 6].

Table 1 compares the performance of currently available malaria diagnostic tests for case management and surveillance. The landscape for malaria diagnosis can be divided into two main groups, POC methods in case management and laboratory-based methods for surveillance [7]. In case management, microscopy and RDTs are the two diagnostic methods that are recommended in primary settings whilst highly sensitive RDTs and molecular diagnostics [polymerase chain reaction (PCR) and loop mediated isothermal amplification (LAMP)] are often used in laboratory settings [8]. While presenting ultra-sensitivity (less than 2 parasites/μL for both Pan and Pf-LAMP) in the field [9, 10], implementing malaria diagnostic tools in the field still requires addressing of several critical challenges such as simplified sample preparation steps, ready to use kits that require no cold
Further, there is no reported literature referring to the use of malaria LAMP as a diagnostic tool in populations, or of being endorsed and procured by any programs or governments. In the meantime, also being less sensitive, conventional RDTs are at much lower cost of approximately 1 $USD per test [12]. Field studies have shown that POC methods such as microscopy and rapid diagnostic tests (RDTs) are effective in low-resource settings (LRS) [10, 13–25].

**Microscopy**

Microscopy is the reference standard for visualization of parasites in blood smears with an analytical sensitivity under normal circumstances approximately tenfold inferior than that of molecular testing [26]. Microscope has been commonly used as a diagnostic tool in peripheral health centres for various reasons, including availability [27]. However, the quality of such diagnosis depends on the availability and skills of trained microscopists, which might not always be available in the LRS, where malaria is endemic.

**Rapid diagnostic tests**

Field studies have confirmed the benefits of introducing RDTs into routine testing such as better case management, improved adherence to test results, and having more rational treatments [28, 29]. Characteristics of current malaria RDTs are summarized in Table 2. Key advantages of RDTs are the ease to use and quick result delivery time (15–20 min). Unlike PCR or microscopy, RDTs detect circulating antigen; therefore they can also be used to detect placental malaria [30]. Diagnosis of malaria in pregnancy is challenging because of placental sequestration, which is specific to \textit{Plasmodium falciparum} infections, can make microscopy detection of parasites difficult.

**Table 1** Characteristics of current malaria diagnostic tools used in case management and surveillance

|                      | LoD (p/µL or ng mL^{-1}) | Sensitivity (%) (95% CI) | Specificity (%) (95% CI) | Cost ($US/test) | Time | Other requirements                      |
|----------------------|--------------------------|--------------------------|--------------------------|-----------------|------|----------------------------------------|
| **Case management**  |                          |                          |                          |                 |      |                                        |
| Microscopy           | Expert: 4–20 [18]        | Depends on microscopist  | ~ 3000                   | 0.12–0.40 [19]  | 60 min | Trained personnel, microscope, Giemsa stain [18] |
|                      | Average: 50–200 [19]    |                          |                          |                 |      |                                        |
| RDTs                 | Existing RDTs: 100 p/µL [22] | > 85% depending on species [19] | > 99% [19] | No need for expensive instrument | 0.55–1.50 [18] | 20 min [20] | Test kit, appropriate storage conditions [18] |
|                      | Latest product: 80 pg/mL for PFAHRP2 [21] |                          |                          |                 |      |                                        |
| **Surveillance**     |                          |                          |                          |                 |      |                                        |
| RDTs                 | Latest product: 80 pg/mL for PFAHRP2 [21] | > 85% depending on species [19] | > 99% [19] | No need for expensive instrument | 0.55–1.50 [18] | 20 min [20] | Test kit, appropriate storage conditions [18] |
| PCR                  | 26 (real-time) [10]      | 100% [23]                | > 99% [10]               | Real-time instrument > 20,000 [25] | 1.5–4.0 [24] | Standard > 6 h | Thermocycler, cold chain, power, reagent grade, water |
|                      | ~ 0.5 to 5.0 [24]        |                          |                          |                 |      |                                        |
| LAMP                 | 47 (real-time) [10]      | 83.3% [22]               | > 99% [22]               | Conventional PCR and LAMP ~ 5000 [25] | 0.40–0.70 [24] | 60 min | Heat source for amplification and DNA extraction |
|                      | ≥ 1 [23]                 | 97.3% [24]               | > 85% [23]               |                 |      |                                        |

\textit{p/µL} parasites/µL, \textit{LoD} limit of detection, \textit{CI} confidence interval

**Table 2** Advantages and disadvantages of current malaria RDTs

| Advantage                  | Disadvantages                                                                 |
|----------------------------|-------------------------------------------------------------------------------|
| Easy to use                | Deletion of the Pfrp2 gene leads to false negative RDTs (particularly in populations in the Amazon region) |
| Low cost                   | Lack of adequate sensitivity for detection of infection in asymptomatic individuals and/or prozone effect |
| Quick result delivery time (< 20 min) | Lack of heat stability when being stored in endemic settings |
| Portable and disposable    | Inability to differentiate non-Pf malaria                                      |
| Require minimal laboratory infrastructure, power or external equipment | Inability to distinguish current and past infections |
| Quick training             | Inability to quantify parasite density, especially for assessing severity of illness or monitoring treatment efficacy |

chain [11]. Further, there is no reported literature referring to the use of malaria LAMP as a diagnostic tool in populations, or of being endorsed and procured by any programs or governments. In the meantime, also being less sensitive, conventional RDTs are at much lower cost of approximately 1 $USD per test [12]. Field studies have shown that POC methods such as microscopy and rapid diagnostic tests (RDTs) are effective in low-resource settings (LRS) [10, 13–25].
Although using the same technology of lateral flow immunoassays, the performance of malaria RDTs varies greatly from brand-to-brand, and lot-to-lot, especially with specimens having low parasite density (<200 parasites/μL). In a collaboration between the Foundation for Innovative New Diagnostics (FIND), the WHO and the Centers for Disease Control and Prevention, 293 malaria RDTs were evaluated from 2008 to 2016 [31]. Most of the evaluated malaria RDTs detect *P. falciparum* histidine-rich protein 2 (PfHRP2) or *P. falciparum* lactate dehydrogenase (PfLDH). In the last round of evaluation, anomalies that interfered with result interpretation were also recorded [31]. The most common anomalies were incomplete clearing and red background, which were observed in 48 and 24% of products. The second most common anomalies were failed migration of liquid, incomplete migration and patchy broken test lines, which occurred in 15, 11 and 11% of the products, respectively.

The performance of lateral flow-based RDTs depends on two main factors: the sensitivity and specificity of antibody-antigen combinations, and the ability to facilitate reliable liquid migration on the nitrocellulose membrane. Much research has focused on new biomarker discovery [32–34], and only limited attention has been paid to reduce limitations imposed by the inhomogeneous migration of liquid across porous nitrocellulose membranes [35].

Figure 1 illustrates how unstructured the flow paths could be in a nitrocellulose membrane [36]. As the migration of liquid occurs in a porous network and is not actively controlled, a number of limitations arise: large volumes of sample needed, accumulation of reagents at the leading edge of the liquid flow, and increased cross-reactivity [37]. It is, therefore, time to consider alternative options to facilitate a more precise liquid migration, hence more accurate test results.

**Promising and alternative technologies for malaria detection**

Table 3 summarizes six major classes of technologies used for detecting malaria and indicates their maturity levels. These technologies are individually reviewed in depth elsewhere [38] and most of them rely on standard concepts using immunoassays [39, 40], molecular diagnostics [41–49] and the visualization of parasites [50–53]. Table 4 provides specifications of some recently entered market malaria diagnostic [38]. Of those market-ready products, four of them are molecular diagnostics, three are immunoassays and one is based on automated microscopy. Several promising proof-of-concepts for the next generation of malaria RDTs are emerging. For example, prototypes have been built to detect the presence of haemozoin in blood sample [54–57]. Haemozoin crystals are produced by *Plasmodium* parasites as a final nontoxic compound of haemoglobin metabolism. In a specific example, a portable light meter was built to image crystalized haemozoin pigment [58]. These pigments are birefringent, so the detection of haemozoin is based on rotating a plane of polarized light through them and observing anisotropic output of the light. The minimum concentration of haemozoin that could be detected with this polarized light system was 15 pg/mL, equivalent to 30 parasites/μL of blood. Applications in the field are to be tested.

Another example utilizes a portable breath analyzer: breaths of malaria-infected patients were found to contain terpenes, a family of aromatic chemicals that are produced by parasites that can further attract mosquitoes [59, 60]. A pilot study in Malawi confirmed that these aromatic compounds could be transported into the lungs and hence could be detected in the exhalation of infected patients [61].

Despite being unquestionably novel, these abovementioned methods of detection still need to prove their practicality for POC in LRS and demonstrate a clinically relevant limit of detection (LOD). For instance, in the breath analyzer, it would be useful to be able to convert the level of terpenes detected in breath into parasite density.

**Specifications for a new generation of malaria RDTs**

Different settings require different target product profiles (TPP) [8]. Unlike previous malaria control campaigns, the key characteristics of malaria elimination efforts are to interrupt endemic transmission and to prevent its re-establishment [62]. The Program for
Appropriate Technology in Health (known as PATH) and FIND are pioneering the development and validation of sensitive rapid tests for mass screening in LRS. They also proposed a TPP for malaria RDTs in elimination settings, stating specific requirements for the ideal rapid tests according to concept of Affordable, Sensitive,
Table 4 Specifications of recently-entered market* technologies for malaria diagnosis. table based on information contained in Ref [38]

| Technology | Product | Developer | Description | Type of detection | Performance | Turn-around time | Sample type | Environmental requirements | Cost per test | Cost per instrument | Power/labour/infrastructure requirements | Result display and storage | Quality control |
|------------|---------|-----------|-------------|------------------|-------------|-----------------|-------------|--------------------------|--------------|----------------------|----------------------------------------|-------------------------|-----------------|
| Microscopy | Parasight | Sight Diagnostics Ldm, 2014 | Automated microscopy suitable for processing of multiple malaria | Slide reading | Under way | n/a | Blood smear | n/a | n/a | n/a | n/a | n/a | n/a |
| Malaria RDTs** | Fio-net | Fio Corporation, 2012 | Universal RDT reader and cloud information services to improve malaria RDT quality assurance and malaria surveillance | Combination of mobile diagnostics (mobile universal reader) with cloud information services | Automated and customising reports Sensitivity and specificity are functions of the RDTs being read | RDT processing time is dependent on manufacturer’s recommendations Data upload within minutes Daily quality control needed | LOD 1.25 parasites/µL | ~ 20 min | 100 µL urine | n/a | n/a | Battery powered Basic 1 day training needed | On screen and web portal | CE marked |
| UMT | Fyodor Biotechnologies, 2015 | A sensitive and specific lateral flow assay detecting novel Plasmodium proteins shed in the urine of febrile malaria patients | Dipstick technology (lateral flow assay) | LOQ 1.25 parasites/µL | Quantitative and qualitative | RDTs processing time is dependent on manufacturer’s recommendations Data upload within seconds | Subject to RDTs manufacturers’ recommendations 5–40 °C | Similar to pre-paid cellphone plans | Battery powered Basic 0.5 day training needed | Customisable | $15.500 | n/a | n/a | n/a |
| Holomic Rapid Diagnostic Reader | Holomic LLC, 2013 | Holomic LLC | Universal RDT reader attachment for smartphones and software to read RDTs and transmit result to a secure cloud information service | Portable, smartphone-based lateral flow immunoassay reader | Quantitative and qualitative | RDTs processing time is dependent on manufacturer’s recommendations Data upload within seconds | Subject to RDTs manufacturers’ recommendations 5–40 °C | Similar to pre-paid cellphone plans | Battery powered Basic 0.5 day training needed | Customisable | $15.500 | n/a | n/a | n/a |
Table 4 (continued)

| Technology          | Product                     | Developer                      | Description                                                                 | Type of detection                  | Performance              | Turn-around time | Sample type      | Sample size | Environmental requirements | Cost per test | Cost per instrument | Power/labour/infrastructure requirements | Result display and storage | Quality control |
|---------------------|-----------------------------|--------------------------------|------------------------------------------------------------------------------|-------------------------------------|--------------------------|------------------|------------------|-------------|--------------------------|--------------|----------------------|------------------------------------------|---------------------------|------------------|
| Nucleic acid detection | LAMP Malaria Diagnostic Kit | Eiken Chemical Ltd and FIND, 2012 | Commercial LAMP test kit containing primers and reagents needed to run assays using benchtop laboratory equipment | Isothermal DNA amplification Fluorescence of visual detection | For pan-LAMP: 97.0% sensitivity For Pf-LAMP: 93.3% sensitivity 85.0% specificity | 60 min            | 30–60 µL blood | Stable for 12 months at < 30 °C | SUSS          | $US10'000 | Electricity (battery-powered possible) 4 days of training required | Turbidimeter and software | CE marked | Positive and negative controls included |
| illumigene LAMP     | Meridian Bioscience         | An automated and compact LAMP technology to qualitatively detect Plasmodium spp. DNA in human whole blood samples | Isothermal DNA amplification | Sensitivity 100% Specificity 89.9% | < 50 min | Human whole blood | Stable for 12 months at 2–30 °C | n/a          | Does not require specialised laboratory equipment | n/a          | CE marked | Does not require specialised laboratory equipment | Turbidimeter and software | CE marked | |
| MicroPCR            | Tulip Group and Bigtec Labs, 2013 | POC real-time quantitative PCR instrument | Fluorescent probe-based real-time PCR | >99% sensitivity and specificity LOD 2 parasites/µL blood | 45–60 min | 100 µL blood | 15–30 °C | $US15 | $US8000 | Battery powered 1–2 days training required | 5000 test results can be stored internally, cloud information available | CE marked | |
| TrueLab             | Molbio, 2013                | A quantitative micro PCR platform containing all equipment and reagents needed for point-of-care applications | Using the proprietary magnetic nanoparticles to capture DNA | n/a | < 60 min | Whole blood | n/a | n/a | A customised micro printer is available | n/a | CE marked | |

* Recently-entered market means products pass the regulatory and policy stage

** G6PD point-of-care tests are not included due to lack of information for popular products. CareStart G6PD RDT (AccessBio) and POC G6PD (PATH) are working on promising products
Specific, User-friendly, Equipment-free and Deliverable (ASSURED) [63]. The desired LOD is 5 parasites/µL or less, or concentration range of 6–12 ng/mL PfHRP2 [63]. For RDT developers it is important to note the caveat of the prozone phenomenon that might prevent detection of high parasite density [64]. Poor specificity could lead to over-treatment, thus depreciation of the intended value of RDTs (from public health perspectives); therefore, the required specificity for effective malaria diagnosis is at least 97% or ideally 99% [63].

Additional requirements for ideal RDTs are suitability and appropriateness for LRS where most malaria cases occur. To make an impact simplicity and affordability are of utmost importance. Simplicity means, the system should be equipment-free and should require very little resources [65]. A simple and automated test could obviate false results caused by user-errors [66]. Affordability is difficult to measure and depends on the cost–benefit equation of a specific situation. Also, tests should be designed to minimize impact of inappropriate storage conditions (2–40 °C) on reagent stability and usability of the devices [67].

Microfluidic technology for malaria POC testing

Microfluidics enable the miniaturization and simplification of complicated analytical processes while consuming less reagents, minimizing waste, and requiring less supporting instrumentation [68]. This stems out from the predictable behaviour of liquids at the microscale where flow is typically laminar. At microscale, minute amounts of liquids can be manipulated using microstructures, such as microvalves, micromixers or micropumps [69]. Low volumes of reagents, fast reaction times, compact and portable platforms are just a few advantages that make microfluidics technology attractive for POC applications [70, 71]. Figure 2 shows several examples demonstrating the archetype of microfluidic-based diagnostics for POC applications, which is an integrated system composed of a disposable unit (where analysis takes place) and a signal acquisition and processing module to process the results. (a) [72], (b) [73], (c) [74].

Currently, microfluidic-based diagnostic devices can be divided into two categories: non-paper-based “traditional” microfluidics and paper-based microfluidics [75, 76]. Research on traditional microfluidics often focuses on miniaturizing conventional techniques. For example, a collection of passive and active mixing elements were designed to facilitate mixing processes on chips [77]. Recent work in developing microfluidic-based diagnostic devices has focused on integrating all necessary elements into stand-alone platforms [78, 79] because such integrated systems can operate without bulky accessories and do not require water, buffer, or a constant supply of electricity [80]. There are many ways to control liquid flows on microfluidic platforms, for instance, acoustic forces, mechanical forces, magnetic forces, as well as capillary and centrifugal forces [81–85]. To satisfy the stringent requirements for LRS, devices based on capillary and centrifugal forces have shown promising results. Table 5 presents some examples of microfluidic-based systems.
that have been designed to detect PfHRP2 and PfLDH antigens or genetic materials from the parasites using on-chip molecular testing, cell deformation mechanism, electrical, optical, and magnetic detections amongst others [54, 58, 79, 81, 86–94].

**Immunodiagnostics on microfluidic platforms for malaria detection**

Standard protocols to perform immunodiagnostics on microfabricated platforms require sample pre-concentration, flow control and detection of biomarkers (analytes

| Application | Concept/detection principle | Biomarker/target | Limit of detection | Performance | Sensitivity (%) | Specificity (%) | Time (min) | Refs |
|-------------|-----------------------------|------------------|-------------------|-------------|----------------|----------------|------------|-----|
| **Molecular analysis** | Paper-based LAMP | P. falciparum, P. vivax, P. pan | 5 p/µL | 61% | 98% | 45 min | [81] |
| Continuous flow PCR | P. falciparum | 2 p/µL | 97.40% | n/a | n/a | 2.5 h | [86] |
| **Cell deformation mechanism** | Inertial focusing | P. falciparum | 2–10 p/µL | n/a | n/a | 400 µL/min | [88] |
| Inertial microfluidics | P. falciparum | 2 cells/min | n/a | [89] |
| Non-inertial lift effect | P. falciparum | Enrichment factor of 4.3 | Throughput 12,000 cells/h | [90] |
| **Electrical detection** | Electrical conductivity of iRBCs | P. falciparum | n/a | n/a | [91] |
| Optofluidic-flow analyser that can measure the optical absorption of RBCs in P. falciparum infected blood sample | P. falciparum | 1712 RBCs/s | n/a | 3 min | [92] |
| Naked-eye screening of in-meso detection of hemozoin crystallites based on birefringence | Hemozoin crystals produced by P. falciparum | n/a | ~ 12 min | [58] |
| **Optical detection** | Visual detection of colored assay spot on a disposable microfluidic card based on a flow-through membrane immunoassay | Malaria PfHRP2 | 10–20 ng/mL | n/a | 1–5 min | [79] |
| Paper-based cartridge containing detection areas for both thin and thick smears | P. falciparum | 100 p/µL | n/a | 30 min | [93] |
| **Magnetic detection** | Cell enrichment microfluidics combined with magnetic relaxometry detection | P. falciparum | 5% parasite density | n/a | 15 min | [54] |
| Detection of hemozoin in iRBCs by magnetic resonance relaxometry | Hemozoin in iRBCs in P. falciparum infections | < 10 p/µL | n/a | Few mins | [94] |

*RBC red blood cell, iRBC infected red blood cell*
and/or parasites). These multi-step protocols can benefit greatly from miniaturization, and in fact, microfluidic-based immunoassays have demonstrated their potential for reliable and accurate performance [95, 96]. Figure 3 presents some examples to illustrate how microfluidics technology can be used to detect malaria by different methods of detection, such as molecular testing, size-based cell sorting, electrical differentiation of healthy and infected red blood cells, optical detection of antigen and magnetic detection of haemozoin. (a) [97], (b) [88], (c) [91], (d) [79], (e) [94].

Sample pre-concentration
Low antigen concentration is a common problem in diagnostic immunoassays and malaria antigen detection is not an exception. To overcome this challenge, several prototypes of analyte concentrator have been developed to enrich biomarkers hence improve LOD. To illustrate how analyte enrichment prior to analysis can improve sensitivity of ELISA, Cheow et al. reported a prototype that can enhance the LOD of prostate-specific-antigen assay up to 1.85 pg/mL [98]. The significant enhancement of 100-fold was achieved by trapping the charged fluorescent product of standard ELISA (analyte-bound enzyme complex) using a multiplex electrokinetic preconcentration technique without modifying the immunobinding process.

Blood is the most common type of specimen for POC testing. However, the cellular components in whole blood often cause non-specific background. To address this problem, a continuous microfluidic device was developed to filter the cells, making plasma available for on-chip analysis [99].

Healthy and *P. falciparum*-infected red blood cells exhibit different ionic permeability of their plasma membrane, with infected cells being more permeable. Therefore, when healthy and infected cells are suspended in a low conductivity medium, infected cells lose internal ions and acquire a different dielectrophoretic mobility than healthy ones [100]. Several groups have developed microfluidic chips using dielectrophoresis and variants of it to separate cells successfully leading to promising prototypes for detecting infected red blood cells thus malaria infections [101–103].

Flow control
Controlling flow on microfabricated devices often introduces a great degree of complexity. For example, a combination of screws, pneumatic and solenoid valves was integrated into a microfluidic platform to actuate flow and control chemical gradients in microchannels [104]. This design might be suitable for laboratory-based tests, but may not lead to robust systems for LRS. Nonetheless, the uses of centrifugation and capillary forces to transport liquids are excellent examples of stand-alone systems [105, 106]. Extensive reviews discussing how to engineer flow path in microscale using capillary and centrifugal forces for POC applications exist [69, 107]. Libraries of microfluidic elements such as valves, mixers and pumps have also been developed [77, 108, 109].

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![Fig. 3](image-url) Examples of microfluidic prototypes for malaria diagnosis using different methods. Reprinted with permission: a from [86], copyright 2016 Wiley–VCH, b from [77], copyright 2014 Royal Society of Chemistry, c from [80] copyright 2014 Elsevier, d from [68] copyright 2012 Royal Society of Chemistry, e from [83] copyright 2014 Springer Nature
Detection
Sensitive detection remains one of the biggest hurdles for clinical diagnosis at the onset of infection. The bottleneck is the limited amount of detectable analytes in a very limited volume of sample. One strategy is to amplify the signal, then convert it into quantitative measurements such as electrical and/or optical signals [96]. The detection strategy is therefore critical for the overall design and fabrication of a device. Optical detection is considered as the ideal read-out for POC applications of microfluidics owing to the simple design and potentially low cost [110, 111]. There are five main categories of optical detection based on the type of generated optical signals: fluorescence, luminescence, absorbance, surface plasmon resonance, and surface-enhanced Raman scattering [112–116]. Detailed discussions about detection strategies for microfluidics systems also exist in the literature [117].

Molecular testing on microfluidic platforms for malaria detection
At the moment, PCR and LAMP are the most sensitive technique for identification of asymptomatic individuals, for example, in 130 clinical samples presenting no parasites based on microscopy, as low as 3.6 × 10⁻⁴ parasite/μL could be identified in 117 samples by a highly sensitive genus-specific quantitative reverse transcriptase real-time PCR (qPCR) [118]. This low LOD was achieved by amplifying and detecting the total nuclear acids of the 185 rRNA genes, which increased the analytical sensitivity of the assay by more than 1 log unit compared to DNA only. However, current applications of PCR and LAMP are still restricted to well-equipped laboratories and thus not suitable for LRS [119]. Miniaturized PCR and/or LAMP is desirable, but developing such devices is a more challenging task than that for biomarkers detection for three reasons: (1) sample pre-treatment is essential for extracting DNA of parasites for downstream analysis, (2) the critical signal amplification step highly depends on temperature control, and (3) robust, low cost, and portable detection techniques are required for remote settings [120].

Sample pre-treatment
The PCR/LAMP process requires isolation of genetic materials from infected cells, pre-concentration, as well as signal amplification and analysis. All steps need to be integrated seamlessly in a closed process to overcome time consuming laboratory-like processing steps. Earlier studies have demonstrated successful prototypes that could sequentially perform cell isolation and lysis for messenger RNA purification [121]. On this device, a unique valving system was designed to facilitate liquid migration and analysis. Microfluidics with “macrofluidics” can also be combined to precisely reconstitute reagents, and automated filling liquids for multiplex PCR technique. A successful story is the Cepheid GeneXpert instrument, where all steps from sample preparation, nucleic acid extraction, to thermal cycling for amplification and eventually detection can be integrated into one platform [122]. A review of microfluidic-based DNA analysis systems is available here [123].

Heating systems
The major challenge of miniaturizing bench-top PCR instruments is the requirement of numerous heating cycles for thermal reactions. To overcome this challenge, micromixers and microchambers were designed to allow thermal reactions to take place rapidly [124]. To speed up DNA amplification by improving thermal transfer through interfaces, microfluidic elements, such as mixers, heaters and temperature controlling units were integrated into glass and silicon substrates [125]. Another strategy to enable different heating regions using continuous flow was investigated using a Peltier element to regulate the temperature for thermal cycling [86]. On this platform, as few as to 2 P. falciparum parasites/μL could be detected. This device offered a simplified sample processing step using desiccated hydrogel, reagents and a camera to detect amplicons. When analysing 188 archived, frozen samples collected in Uganda, this prototype achieved 97.4% sensitivity and 93.8% specificity.

One of the most promising development for stand-alone integrated systems for DNA analysis perhaps was an elegant combination of an exothermic reaction with phase change materials to regulate the heat for thermal cycling [126]. In this prototype, downstream processes such as purification and concentration of sample were integrated seamlessly into the same platform.

Recent work reported by Juul et al. challenged the need of thermal cycling for PCR-like systems by proposing an endogenous enzyme activity detection called rolling-circle enhanced enzyme activity to quantify as little as 1 P. falciparum parasite/μL [87]. The principle of this method is based on using rolling-circle-amplification (RCA) technique to convert a circular DNA template into a 10³ tandem repeat rolling-circle product. In this system, RCA substrates can be processed by the DNA-cleaving enzyme topoisomerase I from Plasmodium parasites, which produces many DNA circles leading to enhanced signal. RCA products can have sizes reaching micrometers, which enable visualization at single molecular level.
**Paper-based microfluidics**

Paper-based microfluidics was proposed by Whitesides and colleagues [127]. Since then, this technology has been growing fast with great promises for global health applications [128]. Unlike its sister products of paper test strips, paper-based microfluidic analytical devices offer well-defined, millimetre-sized microchannels to transport liquids in a controlled manner, yet with low cost for production (< $0.01) [129]. Using hydrophobic “inks” to define areas on hydrophilic paper, it is possible to perform multiple immunodiagnostic assays on the same test strip. To illustrate how complex analytical processes can be simplified and transformed into a paper-based microfluidic device, Pereira et al. integrated concentration and detection steps into a single step assay [130]. The analyte PfLDH in low abundance was first accumulated using a micellar aqueous two-phase system (ATPS). The micellar ATPS consisted in a nonionic Triton X-114 surfactant, which was used to concentrate biomarkers in a sample and enhance the LOD. In this system, a tenfold improved LOD of 10 ng/μL PfLDH was achieved. In an alternative development of a foldable, card-like test device, PfHRP2 could be detected and quantified [131]. The generated signal in presence of PfHRP2 was amplified by gold nanoparticles, yielding a LOD of 1.2 ng/mL PfHRP2, which is four times higher than that of the unamplified case. These studies serve as excellent examples for low cost, non-instrumented analysis systems without compromised performance. Many other innovative approaches to control liquid flows such as selective hydrophobic rendering or origami in which folding of multiple paper layers to trigger reactions were also investigated successfully [132–134].

**Interfacing microfluidic-based analysis with networked mobile devices**

Mobile health applications have rapidly been growing in recent years and there is a trend in interfacing consumer electronics such as smartphones with lateral flow RDTs or microfluidic-based devices [135, 136]. Such combination is expected to deliver increased objectivity of test result interpretation and improved connectivity of the entire healthcare systems. The automation and digitized test results can be more easily combined with other health related parameters and combined with medical decision support systems. User-friendly interfaces, automated result analyses, remote-monitoring and data aggregation, increased storage conditions, and active quality assurance are just a few additional benefits of this approach [137].

In 2008, paper-based microfluidics were integrated with a smartphone camera to perform immunoassays [128]. The camera of the phone was used to take a photograph of the detection zone before and after the deposition of specimen. Since then, many groups have started to develop and enhance capabilities of phone-based low cost diagnostic readers [136]. Table 6 presents an overview of recent work in developing phone-based prototypes that can be used to detect variety of biomarkers for a wide range of diseases with clinically relevant performance. Devices are designed for a broad spectrum of applications, from genetic testing, cancer detection to personalized food allergen monitoring [136, 138–140]. A wide range of strategies are also derived to enhance signal strength, for instance, using Quantum dots, Rayleigh/Mie scatter or gold nanoparticles [141–143]. At present, applications of smartphone-based diagnostics for malaria detection can be divided into two categories: phone-based RDT readers, which provides automatic interpretation of results, and phone-based brightfield microscopes, which allow simple and portable means to visualize parasites in blood samples [138–149].

**Phone-based RDT readers**

A smartphone was used for quantitative reading of the Optimal-IT test, a commercially available malaria RDT with a snap-on unit as reader that is suitable for both Android and iPhone [145]. Images of RDTs were acquired, in either transmission or reflection, and then processed in real time to deliver test results within 10 min. The spatio-temporal information collected by this device can document prevalence of many infectious diseases and would allow efficient tracking of epidemics. Another approach to integrate a custom microfluidic-based immunoassay detecting PfHRP2 with phone-based detection was the development of a microfluidic chip, which can be connected to a phone camera to analyze signals and deliver results in 10 min. The opto-mechanical unit in this case consisted of optical fibers, microfluidic chips and mirrors, and could be easily removed from the back camera of the phone. The principle was to quantify changes in fluorescent intensity upon capturing of PfHRP2 on the sensing region, yielding a LOD of 1 pg/mL of PfHRP2 in 10% diluted blood [144].

**Phone-based bright-field microscope**

Accurate and consistent blood smear reading is challenging to attain in health centres or small clinics in remote regions. A phone-based microscope is a low cost option that can offer enhanced image quality, improved accuracy and user comfort [146, 150]. There are two
simplified imaging techniques suitable for smartphone apps: (1) lens-free holographic imaging, and (2) on-lens devices.

Holography is an image-constructing technique using scattering and interference of light and pixel super-resolution to enhance optical images [151]. An automated lens-less holography was developed with a sufficient field of view of 24 mm² to visualize and capture images of *P. falciparum* [152].

Phone-based microscopy can also be engineered to be a field-ready polarized light microscope without compromised fidelity and resolution [153]. The principle was to detect light birefringence caused by the crystallization of haemoglobin. This field-based, modular microscope could magnify *Plasmodium chabaudi* parasites up to 50 times, gaining a comparable performance compared to conventional polarized microscope. Additional benefits of this prototype are simple operations and low cost per test. Further work using clinical samples could confirm the full potential of this novel phone-based polarized light microscope.

**Conclusion**

Accurate and effective diagnosis is the first step to further pursue efforts to eliminate and reduce the global burden of malaria by 90% in 2030. Current diagnostic methods can detect malaria symptomatic infections, but often miss out asymptomatic cases. The rise in proportion of asymptomatic infections in low transmission areas calls for a new generation of rapid diagnostic tests that can detect the hidden parasite reservoir. Technology is advanced nowadays to (at least theoretically) be able to track down the last parasite carriers. While malaria case management has improved, other causes of fever need to be detected and treated accordingly. Therefore, the ideal RDT should come in as a complete package with ultra-high sensitivity and specificity, meet the ASSURED standards for LRS, and also provide additional diagnostic

| Table 6 Examples of lab-on-a-phone applications |
|-----------------------------------------------|
| **Optical detection** | **Data analysis** | **Signal transduction** | **Target biomarker** | **Sample** | **Platform** | **Performance** | **Refs.** |
| Phone LED and camera + 4 external lenses and mirrors | Mie scattering simulation online | Immunoagglutination (Mie light scattering) | PfHRP malaria biomarker | Human blood | Microbeads | 1 pg/mL–10 ng/mL | [144] |
| Computational power + external optical fiber + LED | Phone application | Fluorescence | Genomic DNA | *Escherichia coli* and *Staphylococcus aureus* | Microfluidics | Comparable to that of commercial PCR |
| Phone camera | Phone app | Colorimetry | HE4 (ovarian cancer biomarker) | Urine | Microchip | 89.5% sensitivity, 90% specificity |
| 2 external LEDs + phone camera | Phone app | Colorimetry | Peanut | Cookies | Sample holder | < 1 ppm |
| External LED + phone camera + additional lens | Phone application | Fluorescence | *Escherichia coli* | Milk, water | Glass capillary | 5–10 cfu/mL |
| External LED and optical fibers | Phone app | Immunochromatography (Mie scatter) | Thyroid stimulating hormone | Human serum | Nitrocellulose test strip | 0.31 mL/U |
| Phone camera + external LED | Computer | Colorimetry | Human IgG | Human IgG sample | Microfluidics, silver deposition | n/a | [143] |
| Snap-on attachment (lens + LEDs) + phone camera | Phone app | Immunochromatography | Malaria biomarkers | Whole blood | Rapid test diagnostic strips | 4 × dilution c.f. RDTs |
| 3 external attachments + lenses + LED + phone camera | Phone application | Fluorescence | Cell count | Blood | Sample holder | 600–2500 white cells/image |
| Phone camera | Phone app | Colorimetry | pH | Test strip | n/a | [147] |
| External LEDs and photodiode | Phone app | Colorimetry | Glucose | Urine | Paper strips | 0–250 mg/dL |
| Snap-on attachments (lens + LED) + phone camera | ImageJ on computer | Fluorescence | Prostate specific antigen (PSA) | Whole blood | Microfluidics | Dynamic range 0.08–60 ng/mL |

LOD 1 pg/mL

LOD 1 pg/mL

LOD 10 mg/dL

LOD 0.4–0.04 ng/mL
capabilities. Microfluidic devices coupled to phone-based readouts offer a unique opportunity to not only reduce the burden of infectious diseases, such as malaria, but also could provide tools for monitoring epidemics and elimination progress on very large scales.

Authors' contributions

NMP drafted the manuscript; NMP and EMD wrote the manuscript with contributions from HPB and WK. All authors read and approved the final manuscript.

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References

1. Cox FEG. History of human parasitology. Clin Microbiol Rev. 2002;15:595–612.
2. WHO. Accelerating progress on HIV, tuberculosis, malaria, hepatitis and neglected tropical diseases: a new agenda for 2016–2030. Geneva: World Health Organization; 2015. p. 64.
3. WHO. World malaria report 2017. Geneva: World Health Organization; 2017.
4. Gates Foundation. From aspiration to action: what will it take to end malaria Seattle: Bill & Melinda Gates Foundation; 2015.
5. WHO. Global technical strategy for malaria 2016–2030. Geneva: World Health Organization; 2015.
6. Mabey D, Peeling RW, Ustianowski A, Perkins MD. Diagnostics for the developing world. Nat Rev Microbiol. 2004;2:231–40.
7. Tangpukdee N, Duangdee C, Wilairatana P, Kruasod S. Malaria diagnosis: a brief review. Korean J Parasitol. 2009;47:93–102.
8. The malERA Consultative Group on Diagnoses and Diagnostics. A research agenda for malaria eradication: diagnoses and diagnostics. PLoS Med. 2011;8:1–10.
9. Vallejo AF, Martinez NL, Gonzalez U, Arevalo-Herrera M, Herrera S. Evaluation of the loop mediated isothermal DNA amplification (LAMP) kit for malaria diagnosis in a vivax endemic settings of Colombia. PLoS Negl Trop Dis. 2015;9:e3453.
10. Cook J, Aydin-Schmidt B, González U, Bell D, Edlund E, Nassor MH, et al. Loop-mediated isothermal amplification (LAMP) for point-of-care detection of asymptomatic low-density malaria parasite carriers in Zanzibar. Malar J. 2015;14:43.
11. Lucia NW, Ndiaye D, Britton S, Udhayakumar V. Expanding the malaria molecular diagnostic options: opportunities and challenges for loop-mediated isothermal amplification tests for malaria control and elimination. Expert Rev Mol Diagn. 2018;18:195–203.
12. WHO. Determining cost effectiveness of malaria rapid diagnostic tests in rural areas with high prevalence. World Health Organization Western Pacific Region. http://www.wpro.who.int/sites/idbPAGE. Accessed 21 May 2018.
13. WHO. New perspective in malaria diagnosis. Geneva: World Health Organization; 2000.
14. Das S, Peck RB, Barney R, Jang IK, Kahn M, Zhu M, et al. Performance of an ultra-sensitive Plasmodium falciparum HRP2-based rapid diagnostic test with recombinant HRP2, culture parasites, and archived whole blood samples. Malar J. 2018;17:118.
15. Das S, Jang IK, Barney B, Peck R, Rek JC, Arah R, et al. Performance of a high-sensitivity rapid diagnostic test for Plasmodium falciparum malaria in asymptomatic individuals from Uganda and Myanmar and naive human challenge infections. Am J Trop Med Hyg. 2017;97:1540–50.
16. Program for Appropriate Technology in Health. Project diameter—enhanced visual parasite detection, 2014.
17. Biocat GmbH. Loop mediated isothermal amplification (LAMP). https://www.biocat.com/genomics/ma-amplification/loop-mediated-isonermal-amplification-lamp. Accessed 21 May 2018.
18. Hathiwala R, Mehta PR, Narang G, Hathiwala S. LED fluorescence microscopy: novel method for malaria diagnosis compared with routine methods. J Infect Public Health. 2016;10:1–5.
19. Wongsrichanalai C, Barcus MJ, Muth S, Supamahardja A, Wernsdorfer WH. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). Am J Trop Med Hyg. 2007;77:119–27.
20. Feleke DG, Tarko S, Hadush H. Performance comparison of CareStart™ HRP2/pLDh combo rapid malaria test with light microscopy in north-western Tigray, Ethiopia: a cross-sectional study. BMC Infect Dis. 2017;17:399.
21. Jimenez A, Rees-Channer RR, Perera R, Gamboa D, Chiodini PL, Gonzalez-U, et al. Analytical sensitivity of current best-in-class malaria rapid diagnostic tests. Malar J. 2017;16:128.
22. UNITAID. Malaria diagnostics technology and market landscape 2015; 2015.
23. Hopkins H, Gonzalez U, Polley SD, Angutoko P, Atekgebra J, Aisimwe C, et al. Highly sensitive detection of malaria parasitemia in a malaria-endemic setting: performance of a new loop-mediated isothermal amplification kit in a remote clinic in Uganda. J Infect Dis. 2013;208:645–52.
24. Cordray MS, Richards-Kortum RR. Emerging nucleic acid-based tests for point-of-care detection of malaria. Am J Trop Med Hyg. 2012;87:223–30.
25. Erdman UK, Kain KC. Molecular diagnostic and surveillance tools for global malaria control. Travel Med Infect Dis. 2008;6:82–99.
26. Hofmann N, Mwingira F, Shekalaghe S, Robinson LJ, Mueller I, Felger I. Ultra-sensitive detection of Plasmodium falciparum by amplification of multi-copy subtelomeric targets. PLoS Med. 2015;12:e1001788.
27. WHO. New perspectives malaria diagnosis. Geneva: World Health Organization; 2000.
28. Ezenzia U, Ndupa SO, Ekunwure OL. Cost benefit analysis of malaria rapid diagnostic test: the perspective of Nigerian community pharmacists. Malar J. 2017;16:7.
29. Bisoffi Z, Sirima SB, Meheus F, Lodesani C, Gobbi F, Angheben A, et al. Analytical performance of the loop-mediated isothermal amplification tests for malaria control and elimination progress on very large scales. Malar J. 2017;16:7.
30. WHO/FIND/CDC. Malaria rapid diagnostic test performance round 1–7 (2008–2016). Geneva: World Health Organization, 2017.
81. Bourquin Y, Reboud J, Wilson R, Zhang Y, Cooper JM. Integrated immunosensor using tunable surface acoustic waves and lens-free detection. Lab Chip. 2011;11:2725–30.
82. Chen C-F, Liu J, Chang C-C, DeVoe DL. High-pressure on-chip mechanical valves for thermoplastic microfluidic devices. Lab Chip. 2009;9:3511–6.
83. Nam J, Huang H, Lim H, Lim C, Shin S. Magnetic separation of malaria-infected red blood cells in various developmental stages. Anal Chem. 2013;85:7316–23.
84. Juncker D, Schmid H, Drechsler U, Wolf H, Wolf M, Michel B, et al. Autonomous microfluidic capillary system. Anal Chem. 2002;74:6139–44.
85. Hugo S, Land K, Madoz M, Kido H. A centrifugal microfluidic platform for point-of-care diagnostic applications. S Afr J Sci. 2014;110:1–7.
86. Taylor BJ, Howell A, Martin KA, Manage DP, Gordy W, Campbell SD, et al. A lab-on-chip for malaria diagnosis and surveillance. Malar J. 2014;13:179.
87. Juul S, Nielsen CJF, Labouriau R, Roy A, Tesauro C, Jensen PW, et al. Droplet microfluidics platform for highly sensitive and quantitative detection of malaria-causing Plasmodium parasites based on enzyme activity measurement. ACS Nano. 2012;6:10676–83.
88. Warkiani ME, Tay AKP, Khoo BL, Xiaofeng X, Han J, Lim CT. Malaria diagnostics using inertial microfluidics. Lab Chip. 2009;7:3330–7.
89. Peng WK, Kong TF, Ng CS, Chen L, Huang Y, Bhagat AAS, et al. Microfluidic concentration and detection of a malaria biomarker. Anal Chim Acta. 2009;652:83–96.
90. Park J, Sunkara V, Kim TH, Hwang H, Cho YK. Lab-on-a-disc for fully integrated multiplex immunosassays. Anal Chem. 2012;84:2133–40.
91. Gervais L, Delamarre E. Toward one-step point-of-care immunodiagnosis using capillary-driven microfluidics and PDM substrates. Lab Chip. 2009;9:3330–2.
92. Strohmeyer O, Keller M, Schwemmer F, Zehnle S, Mark D, von Stetten F, et al. Centrifugal microfluidic platforms: advanced unit operations and applications. Chem Soc Rev. 2015;44:6187–229.
93. Oh KW, Ahn CH. A review of microvalves: J Micromechanics Microengineering. 2006;16:R13–39.
94. Laser DJ, Santiago JG. A review of microumps. J Micromechanics Microengineering. 2004;14:R35–64.
95. Kuswandhi B, Nuriman, Huskens J, Verboom W. Optical sensing systems for microfluidic devices: a review. Anal Chim Acta. 2007;601:141–55.
96. Wu J, Zheng G, Lee LM. Optical imaging techniques in microfluidics and their applications. Lab Chip. 2012;12:3566.
97. Dittrich PS, Manz A. Single-molecule fluorescence detection in microfluidic channels-the Holy Grail in µTAS? Anal Bioanal Chem. 2005;382:1771–82.
98. Mirasoli M, Guardigli M, Micheli E, Rada A. Recent advancements in chemical luminescence-based lab-on-chip and microfluidic platforms for bioanalysis. J Pharm Biomed Anal. 2014;87:36–52.
99. Chin CD, Lakasanopin T, Cheung YK, Steinmiller D, Linder V, Parsa H, et al. Microfluidics-based diagnostics of infectious diseases in the developing world. Nat Med. 2011;17:1015–9.
100. Petryayeva E, Krull UJ. Localized surface plasmon resonance nanostructures, biosassays and biosensing-A review. Anal Chim Acta. 2011;706:24–8.
101. Li M, Cushing SK, Wu N. Plasmon-enhanced optical sensors: a review. Analyst. 2014;140:386–406.
102. Baker CA, Duong CT, Grimley A, Roper MG. Recent advances in microfluidic detection systems. Bioanalysis. 2009;1:967–75.
103. Kamau E, Tolbert LS, Kortepeter L, Pratt M, Nyakoe N, Muringo L, et al. Development of a highly sensitive genus-specific quantitative reverse transcriptase real-time PCR assay for detection and quantification of Plasmodium by amplifying RNA and DNA of the 18S rRNA genes. J Clin Microbiol. 2011;49:2946–33.
104. Batista-dos-Santos S, Raol M, Santos S, Cunha GM, Ribeiro-dos-Santos A. Real-time PCR diagnosis of Plasmodium vivax among blood donors. Malar J. 2012;11:345.
105. Liu P, Mathies RA. Integrated microfluidic systems for high-performance genetic analysis. Trends Biotechnol. 2009;27:572–81.
106. Hong JW, Studer V, Hang G, Anderson WF, Quake SR. A nanoliter-scale nucleic acid processor with parallel architecture. Nat Biotechnol. 2004;22:435–9.
107. Timoney CF, Felder RA. Feature Article Cephed: Expanding the boundaries for practical applications of microinstrumentation and microfluidics. J Assoc Lab Autom. 1998;3:22–6.
108. Brijn E, van Asten A, Tiggelaar R, Gardeniers H. Microfluidic devices for forensic DNA analysis: a review. Biosensors. 2016;6:1–35.
109. Krishnan M, Ugaz VM, Burns MA. PCR in a Rayleigh-Bénard convection cell. Science. 2002;298:793.
110. Burns MA, Johnson BN, Brahmasandra SN, Handique K, Webster JR, Krishnan P, et al. An integrated nanoliter DNA analysis device. Science. 1998;282:484–7.
111. Mcmahon T, Van Zijl PCM, Gilad AA. Instrument-free exothermic heating with phase change temperature control for paper microfluidic devices. Proc SPIE. 2013;27:330–21.
112. Martinez AW, Phillips ST, Butte AJ, Whitesides GM. Patterned paper as a platform for inexpensive, low-volume, portable bioassays. Angew Chem Int Ed. 2007;46:1318–20.
113. Martinez AW, Phillips ST, Carrillo E, Thomas SW III, Sindi H, Whitesides GM. Simple telemedicine for developing regions: camera phones and paper-based microfluidic devices for real-time, off-site diagnosis. Anal Chem. 2008;80:3699–707.
114. Martinez AW, Phillips ST, Whitesides GM, Carrillo E. Diagnostics for the developing world: microfluidic paper-based analytical devices. Anal Chem. 2010;82:3–10.
115. Pereira DY, Chiu RYT, Zhang SCL, Wu BM, Kamei DT. Single-step, paper-based concentration and detection of a malaria biomarker. Anal Chim Acta. 2015;882:83–9.
131. Fu E, Liang T, Spicar-Mihalic P, Houghtaling J, Ramachandran S, Yager P. Two-dimensional paper network format that enables simple multistep assays for use in low-resource settings in the context of malaria antigen detection. Anal Chem. 2012;84:4574–9.

132. Handique K, Gogoi BP, Burke DT, Mastrandelo CH, Burns MA. Microfluidic flow control using selective hydrophobic patterning. In: Proceedings of SPIE—the international society for optical engineering. 1997;3224:185–95.

133. Liu H, Crooks RM. Three-dimensional paper microfluidic devices assembled using the principles of origami. J Am Chem Soc. 2011;133:17564–6.

134. Martinez AW, Phillips ST, Whitesides GM. Three-dimensional paper microfluidic devices fabricated in layered paper and tape. Proc Natl Acad Sci USA. 2008;105:19606–11.

135. Preechaburana P, Suska A, Filippini D. Interfacing diagnostics with consumer electronics. In: Iniewski K, Karlen W, editors. Mob. point-of-care mont. diagnostic device des. Boca Raton: CRC Press; 2014. p. 1–19.

136. Coskun AF, Zhu H, Ozcan A. Three-dimensional paper microfluidic devices fabricated in layered paper and tape. Proc Natl Acad Sci USA. 2011;105:19606–11.

137. Ozcan A. Mobile phones democratize and cultivate next-generation imaging, diagnostics and measurement tools. Lab Chip. 2014;14:3187–94.

138. Stemple CC, Angus SV, Park TS, Yoon J-Y. Smartphone-based optofluidic lab-on-a-chip for detecting pathogens from blood. J Lab Autom. 2014;19:35–41.

139. Muñoz-Yañez O, Díaz-Madrigal M, Fernández-Gracia A, Piqué E, Llorens T, Sancho R, et al.bacterial detection on a cell phone. Microfluidics. 2014;14(1):2678–86.

140. Zhu H, Yaglidere O, Su T-W, Tseng D, Ozcan A. Cost-effective and compact wide-field fluorescent imaging on a cell phone. Lab Chip. 2011;11:315–22.

141. Shen L, Hagen JA, Papautsky I. Point-of-care colorimetric detection with a smartphone. Lab Chip. 2012;12:4240.

142. Coskun AF, Zhu H, Ozcan A, Lab on a Cellphone. In: Karlen W, Iniewski K, editors. Mob. point-of-care mont. diagnostic device des. Boca Raton: CRC Press; 2015. p. 1–19.

143. Scott DB, Tang B, Gao P. Lab-on-a-cellphone for microfluidic applications. J Lab Autom. 2015;19:312–8.

144. Preechaburana P, Suska A, Filippini D. Interfacing diagnostics with consumer electronics. In: Iniewski K, Karlen W, editors. Mob. point-of-care mont. diagnostic device des. Boca Raton: CRC Press; 2014. p. 1–19.

145. Mudanyali O, Dimitrov S, Sikora U, Pasmanabhan S, Navruz I, Ozcan A. Integrated rapid diagnostic test reader platform on a cell phone. Lab Chip. 2012;12:2678–86.

146. Zhu H, Yaglidere O, Su T-W, Tseng D, Ozcan A. Cost-effective and compact wide-field fluorescent imaging on a cell phone. Lab Chip. 2011;11:315–22.

147. Shen L, Hagen JA, Papautsky I. Point-of-care colorimetric detection with a smartphone. Lab Chip. 2012;12:4240.

148. Lee D-S, Jeon BG, Ihm C, Park J-K, Jung MY. A simple and smart telemedicine device for developing regions: a pocket-sized colorimetric reader. Lab Chip. 2011;11:120–6.

149. Barbosa Al, Gehtlot P, Sidappa K, Edwards AD, Reis NM. Portable smartphone quantitation of prostate specific antigen (PSA) in a fluoropolymer microfluidic device. Biosens Bioelectron. 2015;70:5–14.

150. Quin AJ, Andama A, Munabi I, Kivunyaka F. Automated blood smear analysis for mobile malaria diagnosis. In: Karlen W, Iniewski K, editors. Mob. point-of-care mont. diagnostic device des. Boca Raton: CRC Press; 2015. p. 115–31.

151. Mudanyali O, Tseng D, Oh C, Iskman SO, Sencan I, Bishara W, et al. Compact light-weight and cost-effective microscope based on lensless incoherent holography for telemedicine applications. Lab Chip. 2010;10:1417–28.

152. Bishara W, Sikora U, Mudanyali O, Ting-Wei S, Yaglidere O, Lyckhart S, et al. Holographic pixel super resolution in portable lensless op-chip microscopy using a fiber optic array. Lab Chip. 2011;11:1276–9.

153. Pirtill CW, Coté GL. Malaria diagnosis using a mobile phone polarized microscope. Sci Rep. 2015;5:1–13.