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Original article

Performance evaluation of different strategies based on microscopy techniques, rapid diagnostic test and molecular loop-mediated isothermal amplification assay for the diagnosis of imported malaria

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ABSTRACT

Objectives: Malaria is one of most common tropical diseases encountered in travellers and migrants. It requires an urgent and reliable diagnosis considering its potential severity. In this study, performance of five diagnostic assays were evaluated in a nonendemic region and compared prospectively to quantitative PCR (qPCR).

Methods: A prospective study was conducted at Toulouse Hospital from August 2017 to January 2018 and included all patients with initial *Plasmodium* screening. Thin and thick blood smears (TnS, TkS), quantitative buffy coat (QBC), rapid diagnostic tests (RDTs) and commercial loop-mediated isothermal amplification (LAMP) were independently performed on each blood sample and compared to our qPCR reference standard.

Results: The study encompassed 331 patients, mainly returning from Africa. qPCR detected 73 *Plasmodium*-positive samples (including 58 *falciparum*). Individually, LAMP had a 97.3% (71/73) sensitivity, far ahead of TnS (84.9%, 62/73), TkS (86.3%, 63/73), QBC (86.3%, 63/73) and RDT (86.3%, 63/73). RDT demonstrated a high sensitivity for *falciparum* (98.3%, 57/58) but missed all *ovale*, *malariae* and *knowlesi* infections. Specificity was excellent for all techniques (99.6–100%). The most sensitive diagnosis strategies were TnS + RDT (95.9%, 70/73), TnS + LAMP (97.3%, 71/73) and TnS + RDT + LAMP (100%, 73/73), about 10% higher than strategies using exclusively microscopy, TkS + TnS (87.7%, 64/73) or QBC + TnS (87.7%, 64/73). TnS remains necessary for *Plasmodium* species identification and quantification. Adding sequentially TnS only on LAMP-positive samples did not decrease TnS + LAMP strategy sensitivity.

Conclusions: In nonendemic countries, the currently recommended microscopy-based strategies seem unsatisfactory for malaria diagnosis considering RDT and LAMP performance, two rapid and sensitive assays that require limited training. **E. Charpentier, Clin Microbiol Infect 2020;26:115**

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Introduction

Malaria is a potential deadly parasitic infection which requires a rapid and reliable diagnosis [1,2]. In 2015, over 6000 imported malaria cases were recorded in EU/EEA countries, including 2500 in France [3]. However, malaria can be challenging to diagnose in nonendemic countries [2,4], which can lead to misdiagnosis [5] and delays in treatment.

Microscopic diagnostic techniques are the standard methods used to detect *Plasmodium* in most medical laboratories [6] but are time consuming and labour intensive, and their sensitivities may vary greatly depending on the microscopist's experience [7]. Malaria rapid diagnostic test (RDT) based on immunochromatographic techniques are often added to microscopy because they are fast and relatively easy to perform and interpret [8,9]. However, their sensitivities can vary among RDT brands, with unequal results for different *Plasmodium* species [9,10].

Numerous studies have demonstrated that PCR-based assays have the highest sensitivity and specificity for *Plasmodium* detection [11] with a limit of detection generally below one parasite per microlitre [12]. However, these methods do not meet the urgent nature of malaria diagnosis and are limited to specialized parasitology centres because of the technical requirements and equipment needed.

Also based on molecular methods, new commercially available techniques of DNA loop-mediated isothermal amplification (LAMP) were recently developed for malaria diagnosis [13,14]. They have the advantage of a significantly reduced analysis time, now compatible with the 2-hour delay recommended for malaria diagnosis, while having a simple technical process and a high sensitivity [14,15].

Strategies for diagnosing imported malaria vary between medical laboratories [6]. Nevertheless, as a result of the low prevalence of malaria cases in nonendemic countries, highly sensitive, reliable and easy-to-perform methods must be selected [5]. In this context, we needed to evaluate the performance and usefulness of most of the different tests currently available for *Plasmodium* detection, particularly at a time when new LAMP assays are being developed.

The main objectives of our study were to prospectively compare the performance of different strategies used in medical biology laboratories for diagnosing imported malaria based on thin smear (TnS), thick smear (TkS), quantitative buffy coat (QBC), RDT and LAMP; and to determine LAMP's potential place within the existing range of techniques.

Patients and methods

Sample collection

The study was carried out prospectively from August 2017 to January 2018 at the Parasitology Department of Toulouse Teaching Hospital, including all patients with an initial malaria screening. Subjects who had previously received an antimalarial treatment were excluded from the study. Blood samples were obtained only for standard diagnosis on the basis of the physicians' prescriptions. Clinical data were made anonymous for analysis. According to the French public health law [16], protocols of this type do not require approval from an ethics committee and are exempt from the requirement for formal informed consent.

TnS, TkS, QBC, RDT and LAMP assays were performed on each blood sample in the 2 hours after they were obtained, whereas quantitative PCR (qPCR) was performed within 48 hours and constituted our reference test.

Malaria diagnostic techniques

TnS and TkS were prepared, stained and examined microscopically (×1000) by certified operators in accordance with World Health Organization (WHO) recommendations [17,18].

QBC assay (Becton Dickinson, Franklin Lakes, NJ, USA), RDT Palutop + 4 Optima kit (AllDiag, Strasbourg, France) and Alethia assay (ex-Illumigene) LAMP technique (Meridian, Cincinnati, OH, USA) were performed as recommended by the manufacturers. Palutop + 4 Optima detects *P. falciparum*–specific histidine-rich protein 2 (HRP-2) antigen, *P. vivax*–specific lactate dehydrogenase (LDH) antigen and a pan-*Plasmodium* LDH antigen.

DNA extraction was performed from 200 μL of whole blood using the High-Pure PCR Template kit (Roche, Mannheim, Germany) in accordance with the manufacturer's instructions. *Plasmodium* genus qPCR was performed on all samples, and molecular species identification was performed on positive samples as previously described [19].

Statistical analysis

Evaluated combinations of techniques were the ones recommended by French national guidelines or the ones used by medical laboratories [20]. They combine at least one technique aiming to detect *Plasmodium* (TkS, QBC, RDT, LAMP) with TnS for species identification and parasitaemia assessment. Sensitivities and specificities with 95% confidence intervals (CI) were calculated on all samples included in the study using qPCR as the reference test. Negative predictive value and positive predictive value were calculated with a 0.07 prevalence [21].

Results of sensitivity and specificity were compared using a test of equality of proportions. All statistical tests and procedures were performed using the Intercooled Stata 9.2 statistical software package (StataCorp, College Station, TX, USA).

Results

Sample description

From August 2017 to January 2018, a total of 336 initial *Plasmodium* screening (336 patients) were carried out at Toulouse Teaching Hospital, and 331 independent subjects were prospectively included in the study. Five patients were excluded because of limited blood volume ($n = 3$) or receipt of previous antimalarial treatment ($n = 2$). Most patients had come back from Africa (69.2%, 229/331).

The panel encompassed 73 positive samples detected by qPCR. Table 1 summarizes species distributions and origins of *Plasmodium* diagnosed in this study. For positive samples, the context of travel and the clinical presentation are detailed in Supplementary Table S1. Parasite load was evaluated through qPCR quantification cycle (Cq), with a high Cq corresponding to a low amount of *Plasmodium* DNA and vice versa. Cq and parasitaemia are detailed for each positive sample in Supplementary Table S1. Among the six infections with very low parasite load (Cq $\geq$ 30), there were three molecularly identified infections, all of them *falciparum* infections.

Single tests

TnS, TkS, QBC, RDT, LAMP and qPCR assays were performed independently and blindly from one another on each anonymized sample (Table 2). Raw data are detailed in Supplementary Table S1 for positive samples.

Microscopic techniques all had similar sensitivities, with 84.9% (62/73; 95% CI, 81.1–88.8) for TnS and 86.3% (63/73; 95% CI,

Table 1
Plasmodium species distribution and origin

Site	*P. falciparum*	*P. ovale*	*P. vivax*	*P. malariae*	*P. knowlesi*	Coinfections	*Plasmodium* sp.	Total
Africa	56 (76.7)	5 (6.8)	0	2 (2.7)	0	2[a] (2.7)	3[b] (4.1)	68 (93.2)
West Africa	36 (49.3)	4 (5.5)	0	1 (1.4)	0	2[a] (2.7)	3[b] (4.1)	46 (63.0)
Central Africa	18 (24.7)	1 (1.4)	0	1 (1.4)	0	0	0	20 (27.4)
East Africa	2 (2.7)	0	0	0	0	0	0	2 (2.7)
Asia	0	0	1 (1.4)	0	1 (1.4)	0	0	2 (2.7)
South America	1 (1.4)	0	1 (1.4)	0	0	0	0	2 (2.7)
Europe (France)	1 (1.4)	0	0	0	0	0	0	1 (1.4)
Total	58 (79.5)	5 (6.8)	2 (2.7)	2 (2.7)	1 (1.4)	2[a] (2.7)	3[b] (4.1)	73

Data are presented as *n* (%).
[a] Coinfections *P. falciparum*–*P. ovale* ($n = 1$) and *P. falciparum*–*P. malariae* ($n = 1$).
[b] Both PCR studies allowing species identification were negative due to low parasite load.

82.6–90.0) for TkS and QBC (concentration techniques). The sensitivities of these three techniques decreased with lower parasite loads. They detected approximately half of the nine infections with $25 \leq Cq < 30$ cycles and none of the six samples with $Cq \geq 30$ cycles. *Plasmodium* species did not have any influence on test sensitivities.

Palutop + 4 Optima RDT also had a 86.3% sensitivity (63/73; 95% CI, 82.6–90.0). Its sensitivity varied a lot according to *Plasmodium* species: 98.3% (57/58) and 100% (2/2) respectively for *falciparum* and *vivax*, also detecting low *falciparum* infections (2/3 *falciparum* infections with $Cq \geq 30$ cycles), whereas it missed all *ovale, malariae* and *knowlesi* infections.

Alethia malaria LAMP technology had a 97.3% (71/73) global sensitivity (95% CI, 95.5–99.0). It detected all *Plasmodium* species and low-burden infections (4/6 samples with $Cq \geq 30$). Nevertheless, there were 11 uninterpretable results (invalid control), two repeatedly (with a result after the third attempt).

Specificities were excellent for all five tests, with a minimal value of 99.6% (95% CI, 98.9–100) for TnS and LAMP assays (one false-positive result, controlled negative at second attempt/examination).

Test combinations

Combinations of techniques were then analysed as if realized simultaneously and complementarily, depending on strategies used in medical laboratories for malaria diagnosis (Table 3). Microscopic combinations—QBC + TnS and TkS + TnS—both had an 87.7% (64/73) sensitivity (95% CI, 84.1–91.2). RDT + TnS had a sensitivity of 95.9% (70/73; 95% CI, 93.8–98.0), which was significantly higher than microscopic combinations (p 0.0001). Adding TkS or QBC to the RDT + TnS combination did not improve *Plasmodium* detection (sensitivity = 95.9%, 70/73). LAMP + TnS was also more sensitive than microscopic combinations (sensitivity = 97.3%; 71/73; 95% CI, 95.5–99.0; $p < 0.0001$). There was no significant difference between LAMP + TnS or RDT + TnS sensitivities for *Plasmodium* detection (p 0.1663). LAMP + RDT + TnS allowed us to detect all positive samples (100% sensitivity; 95% CI, 100–100) and was significantly more sensitive than LAMP + TnS (p 0.0012). When analysing TnS as a secondary test, performed only on LAMP-positive samples or LAMP/RDT-positive samples (in contrast to the previous simultaneous analysis), the sensitivity did not decrease (sensitivity = 97.3% (71/73) and sensitivity = 100% (73/73), respectively).

Plasmodium species identifications were obtained for 70 of 73 positive samples with our reference PCR techniques. The three remaining were classified as *Plasmodium* sp. (very low parasite loads). Strategies with TnS without RDT correctly identified species of 59 of 70 samples (51/58 *falciparum*). When combining TnS and RDT species-specific antigens (*falciparum* HRP-2 and *vivax* LDH), there were 62 of 70 correct identifications. HRP-2 antigen enabled identification of six more *falciparum* (57/58), but there were three

Table 2
Performance of single malaria assays

Characteristic	TnS	QBC	TkS	RDT	LAMP
Sensitivity					
Global	84.9 (62/73)	86.3 (63/73)	86.3 (63/73)	86.3 (63/73)	97.3 (71/73)
Plasmodium species					
falciparum	87.9 (51/58)	89.7 (52/58)	89.7 (52/58)	98.3 (57/58)	98.3 (57/58)
Non-*falciparum*	90 (9/10)	90 (9/10)	90 (9/10)	20 (2/10)	100 (10/10)
ovale	80 (4/5)	80 (4/5)	80 (4/5)	0 (0/5)	100 (5/5)
vivax	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)
malariae	100 (2/2)	100 (2/2)	100 (2/2)	0 (0/2)	100 (2/2)
knowlesi	100 (1/1)	100 (1/1)	100 (1/1)	0 (0/1)	100 (1/1)
Coinfection	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)
Plasmodium sp.	0 (0/3)	0 (0/3)	0 (0/3)	66.7 (2/3)	66.7 (2/3)
Real-time PCR Cq					
<17	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)
17 ≥ Cq < 25	100 (38/38)	100 (38/38)	100 (38/38)	86.8 (33/38)	100 (38/38)
25 ≥ Cq < 30	44.4 (4/9)	55.6 (5/9)	55.6 (5/9)	66.7 (6/9)	100 (9/9)
≥30	0 (0/6)	0 (0/6)	0 (0/6)	66.7 (4/6)	66.7 (4/6)
Specificity (%)	99.6 (257/258)	100 (258/258)	100 (258/258)	100 (258/258)	99.6 (257/258)
PPV (%)[a]	94.1	100	100	100	94.8
NPV (%)[a]	98.9	99	99	99	99.8

Data are presented as % (*n*/*N*).
Cq, quantification cycle; LAMP, loop-mediated isothermal amplification; QBC, quantitative buffy coat; RDT, rapid diagnostic test; TkS, thick smear; TnS, thin smear; PPV, positive predictive value; NPV, negative predictive value.
[a] Based on malaria prevalence of 0.07.

Table 3
Performance of malaria diagnosis strategies

Characteristic	TkS + TnS	QBC + TnS	RDT + TnS	LAMP + TnS	TkS + TnS + RDT	QBC + TnS + RDT	LAMP + TnS + RDT	LAMP + if positive TnS	LAMP + if positive TnS and RDT	LAMP + RDT + if positive TnS
Sensitivity (%)										
Global	87.7 (64/73)	87.7 (64/73)	95.9 (70/73)	97.3 (71/73)	95.9 (70/73)	95.9 (70/73)	100 (73/73)	97.3 (71/73)	97.3 (71/73)	100 (73/73)
Plasmodium species										
falciparum	91.4 (53/58)	91.4 (53/58)	98.3 (57/58)	98.3 (57/58)	98.3 (57/58)	98.3 (57/58)	100 (58/58)	98.3 (57/58)	98.3 (57/58)	100 (58/58)
Non-*falciparum*	90 (9/10)	90 (9/10)	90 (9/10)	100 (10/10)	90 (9/10)	90 (9/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
ovale	80 (4/5)	80 (4/5)	80 (4/5)	100 (5/5)	80 (4/5)	80 (4/5)	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)
vivax	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)
malariae	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)
knowlesi	100 (1/1)	100 (1/1)	100 (1/1)	100 (1/1)	100 (1/1)	100 (1/1)	100 (1/1)	100 (1/1)	100 (1/1)	100 (1/1)
Coinfection	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)
Plasmodium sp.	0 (0/3)	0 (0/3)	66.7 (2/3)	66.7 (2/3)	66.7 (2/3)	66.7 (2/3)	100 (3/3)	66.7 (2/3)	66.7 (2/3)	100 (3/3)
Real-time PCR Cq										
<17	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)
17 ≥ Cq < 25	100 (38/38)	100 (38/38)	100 (38/38)	100 (38/38)	100 (38/38)	100 (38/38)	100 (38/38)	100 (38/38)	100 (38/38)	100 (38/38)
25 ≥ Cq < 30	66.7 (6/9)	66.7 (6/9)	88.9 (8/9)	100 (9/9)	88.9 (8/9)	88.9 (8/9)	100 (9/9)	100 (9/9)	100 (9/9)	100 (9/9)
≥30	0 (0/6)	0 (0/6)	66.7 (4/6)	66.7 (4/6)	66.7 (4/6)	66.7 (4/6)	100 (6/6)	66.7 (4/6)	66.7 (4/6)	100 (6/6)
Specificity (%)	99.6 (257/258)	99.6 (257/258)	99.6 (257/258)	99.2 (256/258)	99.6 (257/258)	99.6 (257/258)	99.2 (256/258)	99.6 (257/258)	99.6 (257/258)	99.6 (257/258)
PPV (%)[a]	94.3	94.3	94.7	90.2	94.7	94.7	90.4	94.8	94.8	95
NPV (%)[a]	99.1	99.1	99.7	99.8	99.7	99.7	100	99.8	99.8	100
Species identification (%)[b]										
Correct identification	84.3 (59/70)	84.3 (59/70)	88.6 (62/70)	84.3 (59/70)	88.6 (62/70)	88.6 (62/70)	88.6 (62/70)	84.3 (59/70)	88.6 (62/70)	88.6 (62/70)
Misidentification	2.9 (2/70)[c]	2.9 (2/70)[c]	7.1 (5/70)[d]	2.9 (2/70)[c]	7.1 (5/70)[d]	7.1 (5/70)[d]	7.1 (5/70)[d]	2.9 (2/70)[c]	7.1 (5/70)[d]	7.1 (5/70)[d]
Plasmodium sp.	4.3 (3/70)	4.3 (3/70)	1.4 (1/70)	11.4 (8/70)	1.4 (1/70)	1.4 (1/70)	4.3 (3/70)	11.4 (8/70)	2.9 (2/70)	4.3 (3/70)
Undetected[e]	11.4 (6/70)	11.4 (6/70)	2.9 (2/70)	1.4 (1/70)	2.9 (2/70)	2.9 (2/70)	0 (0/70)	1.4 (1/70)	1.4 (1/70)	0 (0/70)

Data are presented as % (*n*/*N*).
Cq, quantification cycle; LAMP, loop-mediated isothermal amplification; QBC, quantitative buffy coat; RDT, rapid diagnostic test; TkS, thick smear; TnS, thin smear; PPV, positive predictive value; NPV, negative predictive value.
[a] Based on a prevalence of malaria of 0.07.
[b] Based exclusively on infections whose species were identified by PCR ($n = 70$).
[c] Only predominant species was identified in coinfections ($n = 2$).
[d] TnS misdiagnoses ($n = 2$) + false positive *vivax* LDH on RDT ($n = 3$).
[e] Undetected *Plasmodium* infection with corresponding strategy.

false-positive results with *vivax* LDH for high *falciparum* parasitaemia malaria. Neither one of these strategies detected the minor species in coinfections. Identification results are detailed in Supplementary Table S1.

Discussion

Early and accurate diagnosis of malaria is essential for both rapid and effective disease management, as misdiagnosis can result in significant morbidity and mortality. In this study, five different techniques and their possible combinations used for diagnosing malaria were evaluated in a hospital located in a nonendemic area.

Microscopic strategies, simultaneously using a concentration technique (QBC or TkS) and TnS, showed significantly lower sensitivities, by almost 10%, than the ones combining TnS with LAMP or RDT. The sensitivities of these two last strategies were not significantly different. The LAMP + RDT + TnS strategy detected all positive samples. Microscopic techniques, including concentration techniques, presented limitations with low-density infections when RDT missed all non-*falciparum/vivax* infections. LAMP technology had an excellent 97.3% sensitivity; the two discrepancies with qPCR concerned samples with parasite loads close to our qPCR limit of detection (estimated at around 0.01 parasite per microlitre). Regarding the identification of *Plasmodium* species, performance was increased when Palutop + 4 Optima was added to TnS because of its sensitive HRP-2 *falciparum*-specific antigen detection.

Currently French national guidelines for diagnosing malaria recommend the simultaneous use of TnS with either TkS or QBC or, as a second choice, if local expertise is lacking, TnS + RDT containing at least HRP-2 and pan-LDH or pan-aldolase antigens, with (if possible) TkS or QBC added if negative [21]. WHO recommends that a diagnosis be performed either by microscopy or antigen detection [22]. In this respect, microscopic diagnosis alone (TkS or QBC with TnS) remains a frequently used strategy in France for malaria diagnosis [6]. However, as observed in our study, its sensitivity is thought to be no higher than 90% in nonendemic countries when compared to PCR [14,23], and it depends on infecting species, geographic origin and the microscopist's experience [23], which can be limited in nonendemic regions. A study by the French National Reference Centre for Malaria showed that 43.7% of 986 city and hospital medical laboratories did not have any cases of malaria in a year [21].

In this study, the most sensitive strategies, based on a combination of tests performed simultaneously, included LAMP (sensitivity = 97.3–100%) or RDT (sensitivity = 95.9–100%), with a maximum 100% sensitivity for the combination including these two tests.

The new molecular LAMP technology has a comparable sensitivity to qPCR while remaining compatible with the urgent nature of malaria diagnosis (result obtained in 45 minutes) and being simple to prepare and interpret. Performance observed in this study is consistent with previous published data comparing LAMP to PCR (97.2–100% sensitivity) [24–26] (Table 4). The 3.3% (11/330) rate of invalid results was comparable to other published data (0 to 5.7%) [24,26–29]. The reasons for these failures are unclear, but because they were solved with a second attempt for nine of 11 samples, it suggests that the operator's training may play a role. Despite its simplicity of use, the LAMP technique—as with other molecular-based diagnostic methods—is susceptible to DNA contamination, and sterile precautions should be taken to avoid false-positive results ($n = 1$ in this study).

RDT has found its place in biomedical laboratories because of its ease of use compared to microscopy (particularly TkS or QBC) and its high performance on the most dreaded *falciparum* species. However, the limitations of RDT on non-*falciparum* species are largely described as well as the performance variability amongst brands [9,29]. As recommended by WHO [30], RDT using HRP-2 antigen, such as Palutop + 4 Optima in this study, should be favoured for its excellent sensitivity on *falciparum* infections, providing its identification in addition.

Nevertheless, neither RDT nor LAMP should be used on its own; they need to be combined with another technique such as TnS to allow for species identification, parasitaemia assessment and non-*falciparum Plasmodium* detection for RDT. However, given LAMP's high negative predictive value for all species, this technology could be used as a single test for *Plasmodium* detection, as already suggested by others [24,25,28,29]. In the event of a positive result, TnS (with or without RDT) would be required to determine the *Plasmodium* species and parasitaemia. This approach can save time because most suspected malaria cases are actually negative in nonendemic areas (78% in this study). As observed in this study, the addition of TnS only in cases of LAMP positivity did not affect the overall sensitivity of the malaria diagnosis.

Moreover, RDT and LAMP respectively detect *Plasmodium* antigen and DNA, and tests may thus remain positive for a few weeks after effective infection treatment. In case of negative microscopy, it could lead to difficulties in interpretation—hence the need for contextual information such as previous antimalarial treatment. These two techniques are also not useful for parasitaemia follow-up.

Table 4
Performance of Alethia (ex-Illumigene) LAMP assay

Study	Area of study	Reference standard method	Type of study	Alethia LAMP assay	Total no. of patients	No. malaria positive	Sensitivity	Specificity	PPV	NPV
Lucchi 2016 [27]	E	PCR	Retro/Prosp	M	209	144	97.2	93.8	—	—
				MP	209	144	97.2	87.7	—	—
Rypien 2017 [28]	NE	Microscopy	Retro	M	139	75	97.3	93.8	45.2[a]	99.8[a]
				MP	132	73	100	91.5	38.2[a]	100[a]
Ponce 2017 [29]	NE	Microscopy	Prosp	MP	299	79	100	93.64	84.95	100
		PCR	Prosp	MP	299	85	100	98.13	95.51	100
De Koninck2017 [24]	NE	Microscopy/RDT/PCR	Retro	M	108	74	100	100	—	—
Frickmann 2018 [25]	NE	Microscopy/PCR	Prosp	M	1000	238	98.7	99.6	98.7	99.6
Cheaveau2018 [26]	NE	Microscopy/PCR	Prosp	M	298	25	100	100	100[b]	100[b]
Charpentier (this report)	NE	PCR	Prosp	M	331	73	97.3	99.6	94.8[c]	99.7[c]

E, malaria endemic area; M, Meridian *Alethia* Malaria; MP, Meridian *Alethia* Malaria Plus; NE, malaria nonendemic area; Prosp, prospective study (fresh samples); Retro, retrospective study (frozen samples); PPV, positive predictive value; NPV, negative predictive value.

[a] Based on prevalence of malaria of 0.05.

[b] Based on prevalence of malaria of 0.044.

[c] Based on prevalence of malaria of 0.07.

The RDT + TnS combination has some limitations on non-*falciparum* infections with low densities (a few cases in our study). It is also more time-consuming than the strategies LAMP + if positive TnS or LAMP + RDT + if positive TnS because it requires a TnS for both positive or negative samples. Nevertheless, it is less expensive than the cost of the Alethia malaria assay (US$21 to $27 per single test), even if cost-effectiveness studies are required to fully answer this question.

The main strengths of this study are its prospective nature with a blinded and independent execution of each technique in standardized laboratory conditions and systemic qPCR as the reference standard. Rigorous patient selection was performed; we only included individuals with initial malaria screening and excluded patients with confirmed treated malaria to avoid bias due to circulating DNA without active *Plasmodium*.

However, these results are limited to the context of imported malaria diagnosis in laboratories with a predominant African epidemiology, where *falciparum* is the most common species. Nevertheless, this distribution is consistent with the reported epidemiology of imported malaria in France and Europe [4,30]. Moreover, there were few non-*falciparum* infections with low parasite loads, possibly favouring the RDT + TnS strategy. It should also be noted that these conclusions are related to one brand of LAMP assay (Alethia (ex-Illumigene) Malaria, Meridian) and RDT (Palutop + 4 Optima, AllDiag) and must be interpreted cautiously for other tests of the same type. Moreover, this evaluation was carried out in a specialized parasitology laboratory that had well-trained staff, which could possibly lead to an overestimation of microscopy performance compared to general biomedical laboratories.

In conclusion, this study showed that malaria diagnosis strategies based on microscopic techniques alone are not the most sensitive options—even more critically in nonendemic countries where the operator's experience may be limited. However, a simultaneous RDT + TnS strategy and LAMP with or without RDT followed by TnS in positive samples are two good options. Indeed, LAMP assay and RDT are two sensitive techniques that are easier for nonexperts to perform, thus ensuring a rapid and reliable detection of *Plasmodium* (*falciparum* for RDT). In both cases, some microscopy expertise remains necessary at least to determine species and parasitaemia. The current international recommendations [8,21], still based on microscopy and RDTs, could evolve towards integration of LAMP molecular assay in initial malaria screening. New guidelines published by the French Infectious Diseases Society [30] recommend the use of concentration techniques or LAMP technology first, followed by TnS if positive, or simultaneous RDT + TnS as a second-choice alternative.

Transparency declaration

All authors report no conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2019.05.010.

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