



HAL
open science

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

E. Charpentier, E. Benichou, A. Pagès, P. Chauvin, J. Fillaux, A. Valentin, H. Guegan, E. Guemas, A.-S. Salabert, C. Armengol, et al.

► To cite this version:

E. Charpentier, E. Benichou, A. Pagès, P. Chauvin, J. Fillaux, et al.. 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. *Clinical Microbiology and Infection*, 2020, 26, pp.115-121. 10.1016/j.cmi.2019.05.010 . hal-03488977

HAL Id: hal-03488977

<https://hal.science/hal-03488977>

Submitted on 7 Mar 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

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

5
6 **Elena Charpentier^{1,2}, Etienne Benichou¹, Arnaud Pagès^{3,4}, Pamela Chauvin¹, Judith**
7 **Fillaux¹, Valentin Alexis¹, Hélène Guegan¹, Emilie Guemas¹, Anne-Sophie Salabert^{5,6},**
8 **Catherine Armengol¹, Sandie Menard², Sophie Cassaing¹, Antoine Berry^{1,2} and Xavier**
9 **Iriart^{1,2}**

10
11 ¹Department of Parasitology-Mycolology, Toulouse University Hospital, Toulouse, France

12 ²Centre for Physiopathology of Toulouse Purpan (CPTP), University of Toulouse, CNRS, INSERM, UPS,
13 Toulouse, France

14 ³Department of Pharmacy, Toulouse University Hospital, Toulouse, France

15 ⁴UMR 1027, Inserm, UPS University of Toulouse III, Toulouse, France

16 ⁵ToNIC, Toulouse Neuroimaging Center, University of Toulouse, Inserm, UPS, Toulouse, France.

17 ⁶Department of Radiopharmacy, Toulouse University Hospital, Toulouse, France
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36

37 Reprints or correspondence: Xavier Iriart (Email: iriart.x@chu-toulouse.fr) and Eléna
38 Charpentier (Email: charpentier.e@chu-toulouse.fr), Service de Parasitologie Mycologie,
39 Centre Hospitalier Universitaire de Toulouse, Hôpital Purpan, Institut Fédératif de biologie
40 (IFB), 330 avenue de Grande Bretagne, TSA 40031, 31059 Toulouse Cedex 9, France.
41

1 **ABSTRACT**

2
3 **OBJECTIVES:** Malaria is one of most common tropical diseases encountered in travellers
4 and migrants. It requires an urgent and reliable diagnosis considering its potential severity. In
5 this study, performance of five diagnostic assays were evaluated, in a non-endemic region and
6 compared prospectively to quantitative PCR (qPCR).

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

12 **RESULTS:** The study encompassed 331 patients, mainly returning from Africa. QPCR
13 detected 73 *Plasmodium*-positive samples (including 58 *falciparum*). Individually, LAMP had
14 a 97.3% (71/73) sensitivity, far ahead of TnS (84.9%, 62/73), TkS (86.3%, 63/73), QBC
15 (86.3%, 63/73) and RDT (86.3%, 63/73). RDT demonstrated a high sensitivity for *falciparum*
16 (98.3%, 57/58) but missed all *ovale*, *malariae* and *knowlesi* infections. Specificity was
17 excellent for all techniques (99.6%-100%). The most sensitive diagnosis strategies were
18 TnS+RDT (95.9%, 70/73), TnS+LAMP (97.3%, 71/73) and TnS+RDT+LAMP (100%,
19 73/73), about 10% higher than strategies using exclusively microscopy, TkS+TnS (87.7%,
20 64/73) or QBC+TnS (87.7%, 64/73). TnS remains necessary for *Plasmodium* species
21 identification and quantification. Adding sequentially TnS only on LAMP-positive samples
22 did not decrease TnS+LAMP strategy sensitivity.

23 **CONCLUSION:** In non-endemic countries, the currently recommended microscopy-based
24 strategies seem unsatisfactory for malaria diagnosis considering RDT and LAMP
25 performance, two rapid sensitive assays that require limited training.

26
27
28
29 **RUNNING TITLE:** Performance evaluation of five malaria diagnostic assays in non-
30 endemic regions

31
32 **KEYWORDS:** Malaria, diagnosis, microscopy, LAMP, loop-mediated isothermal
33 amplification, Illumigene, Alethia, QBC, quantitative buffy coat, RDT, rapid diagnostic test,
34 Palutop+4, thin smear, thick smear

1 INTRODUCTION

2
3 Malaria is a potential deadly parasitic infection which requires a rapid and reliable diagnosis
4 (1, 2). In 2015, over 6,000 imported malaria cases were recorded in EU/EEA countries,
5 including 2,500 in France (3). However, malaria can be challenging to diagnose in non-
6 endemic countries (2, 4), which can lead to misdiagnosis (5) and delays in treatment.

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

13 Numerous studies have demonstrated that PCR-based assays have the highest sensitivity and
14 specificity for *Plasmodium* detection (11) with a limit of detection (LOD) generally below
15 one parasite/ μ L (12). However, these methods do not meet the urgent nature of malaria
16 diagnosis and are limited to specialised parasitology centres due to their technical
17 requirements and the equipment needed.

18 Also based on molecular methods, new commercially available techniques of DNA loop-
19 mediated isothermal amplification (LAMP) were recently developed for malaria diagnosis
20 (13, 14). They have the advantage of a significantly reduced analysis time, now compatible
21 with the 2-hour delay recommended for malaria diagnosis, while having a simple technical
22 process and a high sensitivity (14, 15).

23 Strategies for diagnosing imported malaria vary between medical laboratories (6).
24 Nevertheless, due to the low prevalence of malaria cases in non-endemic countries, highly
25 sensitive, reliable and easy-to-perform methods must be selected (5). In this context, we
26 needed to evaluate the performance and usefulness of most of the different tests currently
27 available for *Plasmodium* detection, particularly at a time when new LAMP assays are being
28 developed.

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

33 34 35 PATIENTS AND METHODS

36 37 *Samples collection*

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

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

49 50 *Malaria diagnostic techniques*

1 TnS and TkS were prepared, stained and examined microscopically (x1000) by certified
2 operators in accordance with World Health Organization (WHO) recommendations (17, 18).
3 QBC assay (Becton Dickinson, Franklin Lakes, N.J.), RDT PALUTOP®+4 Optima kit (All.
4 Diag, Strasbourg, France) and Alethia assay (ex-illumigene) LAMP technique (Meridian,
5 Cincinnati, OH, USA) were performed as recommended by the manufacturers.
6 PALUTOP®+4 detects *P. falciparum* specific-HRP-2 antigen, *P. vivax* specific-LDH antigen
7 and a pan-*Plasmodium* LDH antigen.
8 DNA extraction was performed from 200µL of whole blood using High-Pure PCR Template
9 kit (Roche, Mannheim, Germany) in accordance with the manufacturer's instructions.
10 *Plasmodium* genus qPCR was performed on all samples and molecular species identification
11 were performed on positive samples as previously described (19).
12

13 ***Statistical analysis***

14 Evaluated combinations of techniques were the ones recommended by French national
15 guidelines or the ones used by medical laboratories (20). They combine at least one technique
16 aiming to detect *Plasmodium* (TkS, QBC, RTD, LAMP) with TnS for species identification
17 and parasitaemia assessment. Sensitivities and specificities with 95% confidence intervals
18 (CI95) were calculated on all samples included in the study using qPCR as the reference test.
19 NPV and PPV were calculated with a 0.07 prevalence (21).

20 Results of sensitivity and specificity were compared using a test of equality of proportions.
21 All statistical tests and procedures were performed using the Intercooled Stata 9.2 statistical
22 package (StataCorp, College Station, TX).
23

24 **RESULTS**

25 ***Samples description***

26 From August 2017 to January 2018, 336 initial *Plasmodium* screening (336 patients) were
27 carried out at Toulouse Teaching Hospital and 331 independent subjects were prospectively
28 included in the study. Five patients were excluded due to a limited blood volume (n=3) or a
29 previous antimalarial treatment received by the subjects (n=2). Most patients had come back
30 from Africa (69.2%, 229/331).
31

32 The panel encompassed 73 positive samples detected with qPCR. Table 1 summarises species
33 distributions and origins of *Plasmodium* diagnosed in this study. For positive samples, the
34 context of travel and the clinical presentation are detailed in supplemental table 1. Parasite
35 load was evaluated through qPCR quantification cycle (Cq), high Cq corresponding to low
36 amount of *Plasmodium* DNA and the other way round. Cq and Parasiteamia are detailed for
37 each positive sample in supplemental table 1. Among the 6 infections with very low
38 parasitological load ($Cq \geq 30$), there were 3 molecularly identified infections, all of them being
39 *falciparum* infections.
40

41 ***Single tests***

42 TnS, TkS, QBC, RDT, LAMP and qPCR assays were performed independently and blindly
43 from one another on each anonymised sample (Table 2). Raw data are detailed in
44 supplemental table 1 for positive samples.

45 Microscopic techniques all had similar sensitivities with 84.9% (62/73) CI95[81.1%-88.8%]
46 for TnS and 86.3% (63/73) CI95[82.6%-90.0%] for TkS and QBC (concentration techniques).
47 The sensitivities of these three techniques decreased with lower parasite loads. They detected
48 approximately half of the nine infections with $25 \leq Cq < 30$ cycles and none of the six samples
49 with $Cq \geq 30$ cycles. *Plasmodium* species did not have any influence on their sensitivities.
50

1 Palutop+4 RDT also had a 86.3% sensitivity (63/73) CI95[82.6%-90.0%]. Its sensitivity
2 varied a lot according to *Plasmodium* species: 98.3% (57/58) and 100% (2/2) respectively for
3 *falciparum* and *vivax*, also detecting low *falciparum* infections (2/3 *falciparum* infections with
4 $Cq \geq 30$ cycles), whereas it missed all *ovale*, *malariae* and *knowlesi* infections.

5 *Alethia* malaria LAMP technology had a 97.3% global sensitivity CI95[95.5%-99.0%]
6 (71/73). It detected all *Plasmodium* species and low burden infections (4/6 samples with $Cq \geq$
7 30). Nevertheless, there were eleven uninterpretable results (invalid control), two repeatedly
8 (with a result after the third attempt).

9 Specificities were excellent for all five tests with a minimal value of 99.6% (CI95[98.9%-
10 100%]) for TnS and LAMP assays (one false positive result, controlled negative at second
11 attempt/examination).

12 **Test combinations**

13 Combinations of techniques were then analysed as if realized simultaneously and
14 complementarily, depending on strategies used in medical laboratories for malaria diagnosis
15 (Table 3). Microscopic combinations, QBC+TnS and Tks+TnS, had both an 87.7% (64/73)
16 sensitivity (CI95[84.1%-91.2%]). RDT+TnS had a sensitivity of 95.9% (70/73) CI95[93.8%-
17 98.0%], which was significantly higher than microscopic combinations ($p=0.0001$). Adding
18 Tks or QBC to the RDT+TnS combination did not improve *Plasmodium* detection
19 ($Se=95.9\%$, 70/73). LAMP+TnS was also more sensitive than microscopic combinations,
20 $Se=97.3\%$ (71/73) CI95[95.5%-99.0] ($p<0.0001$). There was no significant difference
21 between LAMP+TnS or RDT+TnS sensitivities for *Plasmodium* detection ($p=0.1663$).
22 LAMP+RDT+TnS allowed us to detect all positive samples (100% sensitivity CI95[100%-
23 100%]) and was significantly more sensitive than LAMP+TnS ($p=0.0012$). When analyzing
24 TnS as a secondary test, performed only on LAMP-positive samples or LAMP/RDT-positive
25 samples (in contrast with the previous simultaneous analysis), the sensitivity did not decrease
26 [$Se=97.3\%$ (71/73) and $Se=100\%$ (73/73), respectively].

27 *Plasmodium* species identifications were obtained for 70/73 positive samples with our
28 reference PCR techniques. The 3 remaining were classified as *Plasmodium sp* (very low
29 parasite loads). Strategies with TnS without RDT, correctly identified species of 59/70
30 samples (51/58 *falciparum*). When combining TnS and RDT species-specific antigens
31 (*falciparum*-HRP-2 and *vivax*-LDH), there were 62/70 correct identifications. HRP2 antigen
32 enabled to identify 6 more *falciparum* (57/58) but there were 3 false positive results with
33 *vivax*-LDH for high *falciparum* parasitaemia malaria. Neither one of these strategies detected
34 the minor species in co-infections. Identification results are detailed in supplemental table 1.
35
36
37

38 **DICUSSION**

39
40 Early and accurate diagnosis of malaria is essential for both rapid and effective disease
41 management, as misdiagnosis can result in significant morbidity and mortality. In this study,
42 five different techniques and their possible combinations used for diagnosing malaria were
43 evaluated in a hospital located in a non-endemic area.

44 Microscopic strategies, using simultaneously a concentration technique (QBC or Tks) and
45 TnS showed significantly lower sensitivities, by almost 10%, than the ones combining TnS
46 with LAMP or RDT. The sensitivities of these two last strategies were not significantly
47 different. LAMP+RDT+TnS strategy detected all positive samples. Microscopic techniques,
48 including concentration techniques, presented limitations with low-density infections when
49 RDT missed all non-*falciparum/vivax* infections. LAMP technology had an excellent 97.3%
50 sensitivity, the two discrepancies with qPCR concerning samples having parasite loads close

1 to our qPCR LOD (estimated at around 0.01 parasite/ μ L). Regarding the identification of
2 *Plasmodium* species, performance was increased when Palutop+4 was added to TnS because
3 of its sensitive HRP-2 *falciparum*-specific antigen detection.
4 Currently, French national guidelines for diagnosing malaria recommend the simultaneous
5 use of TnS with either TkS or QBC or, as a second choice, if local expertise is lacking,
6 TnS+RDT containing at least HRP-2 and pan-LDH or pan-aldolase antigens, with if possible
7 TkS or QBC added if negative (21). WHO recommends that a diagnosis be performed either by
8 microscopy or antigen detection (22). In this respect, microscopic diagnosis alone (TkS or
9 QBC with TnS) remains a frequently used strategy in France for malaria diagnosis (6).
10 However, as observed in our study, its sensitivity is thought to be no higher than 90% in non-
11 endemic countries when compared with PCR (14, 23) and depends on infecting species,
12 geographic origin, and the microscopist's experience (23) which can be limited in non-
13 endemic regions. A study by the French National Reference Centre for Malaria showed that
14 43.7% of 986 city and hospital medical laboratories did not have any cases of malaria in a
15 year (21).
16 In this study, the most sensitive strategies based on a combination of tests simultaneously
17 performed, included LAMP (Se=97.3-100%) or RDT (Se=95.9-100%) with a maximum
18 100% sensitivity for the combination including these 2 tests.
19 The new molecular LAMP technology has a comparable sensitivity to qPCR while remaining
20 compatible with the urgent nature of malaria diagnosis (result obtained in 45 minutes) and
21 being simple to prepare and interpret. Performance observed in this study is consistent with
22 previous published data comparing LAMP to PCR (97.2-100% sensitivity) (24–26) (Table 4).
23 The 3.3% (11/330) rate of invalid results was comparable to other published data (0 to 5.7%)
24 (24, 26–29). The reasons for these failures are unclear but as they were solved with a second
25 attempt for 9/11 samples, it suggests that the operator's training may have a role to play.
26 Despite its simplicity of use, LAMP technique - as with other molecular-based diagnostic
27 methods - is susceptible to DNA contamination and "sterile" precautions should be taken to
28 avoid false positive results (one in this study).
29 RDT has found its place in biomedical laboratories because of its ease of use compared to
30 microscopy (particularly TkS or QBC) and its high performance on the most dreaded
31 *falciparum* species. However, the limitations of RDT on non-*falciparum* species are largely
32 described as well as the performance variability amongst brands (9, 29). As recommended by
33 WHO (30), RDT using HRP-2 antigen such as Palutop+4 in this study, should be favoured for
34 its excellent sensitivity on *falciparum* infections, providing its identification in addition.
35 Nevertheless, neither RDT or LAMP should be used on its own and needs to be combined
36 with another technique such as TnS to allow for species identification, parasitaemia
37 assessment and non-*falciparum Plasmodium* detection for RDT. However, given LAMP high
38 negative predictive value for all species, this technology could be used as a single test for
39 *Plasmodium* detection as already suggested by others (24, 25, 28, 29). In the event of a
40 positive result, TnS (+/-RDT) would be required to determine *Plasmodium* species and
41 parasitaemia. This approach can save time as most of suspected malaria cases are actually
42 negative in non-endemic areas (78% in this study). As observed in this study, the addition of
43 TnS, only in case of LAMP positivity, did not affect the overall sensitivity of malaria
44 diagnosis.
45 Moreover, RDT and LAMP respectively detect *Plasmodium* antigen and DNA, thus remain
46 positive for a few weeks after an effective infection treatment. In case of negative
47 microscopy, it could lead to difficulties in interpretation, hence the need for contextual
48 information such as previous antimalarial treatment. These two techniques are also not useful
49 for parasitaemia follow-up.

50

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

7 The main strengths of this study are its prospective nature with a blind and independent
8 execution of each technique in standardised laboratory conditions and a systemic qPCR as
9 gold standard. Rigorous patient selection was performed, only including individuals with
10 initial malaria screening, and excluding patients with confirmed treated malaria to avoid bias
11 due to circulating DNA without active *Plasmodium*.

12 However, these results are limited to the context of imported malaria diagnosis, in laboratories
13 with a predominant African epidemiology where *falciparum* is the most common species.
14 Nevertheless, this distribution is consistent with the reported epidemiology of imported
15 malaria in France and Europe (4, 30). Moreover, there were few non-*falciparum* infections
16 with low parasite loads, possibly favouring RDT+TnS strategy. It should also be noted that
17 these conclusions are related to one brand of LAMP assay (*Alethia* [ex-*Illumigene*] Malaria,
18 Meridian) and RDT (Palutop+4 optima, ALLDIAG SA) and must be interpreted cautiously
19 for other tests of the same type. Moreover, this evaluation was carried out in a specialised
20 parasitology laboratory that had well-trained staff, which could possibly lead to an
21 overestimation of microscopy performance compared to general biomedical laboratories.
22

23 In conclusion, this study showed that malaria diagnosis strategies based on microscopic
24 techniques alone are not the most sensitive options, even more critically in non-endemic
25 countries where the operator's experience may be limited. On the other hand, simultaneous
26 RDT+TnS strategy and LAMP+/-RDT followed by TnS in positive samples are two good
27 options. Indeed, LAMP assay and RDT are two sensitive techniques that are easier for non-
28 expert insuring a rapid and reliable detection of *Plasmodium* (*falciparum* for RDT). In both
29 cases, some microscopy expertise remains necessary at least to determine species and
30 parasitaemia. The current international recommendations (8, 21), still based on microscopy
31 and RDTs, could evolve towards integration of LAMP molecular assay in initial malaria
32 screening. New guidelines published by the French Infectious Diseases Society (30)
33 recommend the use of concentration techniques or LAMP technology first, followed by TnS
34 if positive, or simultaneous RDT+TnS as a second choice alternative.
35
36
37

38 **ACKNOWLEDGMENTS**

39 The authors gratefully acknowledge the staff at the molecular and morphology units of the
40 Parasitology-Mycology Department, the biology students at the Parasitology-Mycology
41 Department and Solten company for revision of the English text.
42
43

44 This study received a non-financial support from Meridian inc. which provided the *Alethia*
45 instrument (incubator/reader) and part of *Alethia* malaria kit for the study but none of the
46 Meridian staff were involved in the study procedure, data analysis or manuscript writing.

47 No further funding was received.
48
49

1 **TABLES**

2
3
4
5
6

Table 1: Plasmodium species distribution and origin

	<i>P. falciparum</i>	<i>P. ovale</i>	<i>P. vivax</i>	<i>P. malariae</i>	<i>P. knowlesi</i>	Co-infections	<i>Plasmodium sp.</i>	Total
Africa	56 (76.7%)	5 (6.8%)	0	2 (2.7%)	0	2 ¹ (2.7%)	3 ² (4.1%)	68 (93.2%)
West Africa	36 (49.3%)	4 (5.5%)	0	1 (1.4%)	0	2 ¹ (2.7%)	3 ² (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%)	2¹ (2.7%)	3³ (4.1%)	73

7 ¹Co-infections *P. falciparum*-*P. ovale* (n=1) and *P. falciparum*-*P. malariae* (n=1)

8 ²Both PCR allowing species identification were negative due to a low parasite load

9
10
11
12

1
2
3

Table 2: Performance of single malaria assays

	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)
<i>Plasmodium</i> species					
<i>falciparum</i>	87.9 (51/58)	89.7 (52/58)	89.7 (52/58)	98.3 (57/58)	98.3 (57/58)
non- <i>falciparum</i>	90 (9/10)	90 (9/10)	90 (9/10)	20 (2/10)	100 (10/10)
<i>ovale</i>	80 (4/5)	80 (4/5)	80 (4/5)	0 (0/5)	100 (5/5)
<i>vivax</i>	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)
<i>malariae</i>	100 (2/2)	100 (2/2)	100 (2/2)	0 (0/2)	100 (2/2)
<i>knowlesi</i>	100 (1/1)	100 (1/1)	100 (1/1)	0 (0/1)	100 (1/1)
Coinfections	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)
<i>Plasmodium sp.</i>	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 (%)¹	94.1	100	100	100	94.8
NPV (%)¹	98.9	99	99	99	99.8

4
5
6
7
8

TnS: thin smear, QBC: quantitative buffy coat, TkS: thick smear, RDT: rapid diagnostic test, LAMP: Loop-mediated isothermal amplification

¹Based on a prevalence of malaria of 0.07

Table 3: Performance of Malaria diagnosis strategies

1
2

3

	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										
<i>falciparum</i>	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- <i>falciparum</i>	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)
<i>ovale</i>	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)
<i>vivax</i>	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)
<i>malariae</i>	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)
<i>knowlesi</i>	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)
Co-infections	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)
<i>Plasmodium sp.</i>	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 (%)¹										
	94.3	94.3	94.7	90.2	94.7	94.7	90.4	94.8	94.8	95
NPV (%)¹										
	99.1	99.1	99.7	99.8	99.7	99.7	100	99.8	99.8	100
Species identification (%)²										
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) ³	2.9 (2/70) ³	7.1 (5/70) ⁴	2.9 (2/70) ³	7.1 (5/70) ⁴	7.1 (5/70) ⁴	7.1 (5/70) ⁴	2.9 (2/70) ³	7.1 (5/70) ⁴	7.1 (5/70) ⁴
<i>Plasmodium sp.</i>	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 ⁵	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)

4 TkS: thick smear, TnS: Thin Smear, RDT: rapid diagnostic test, LAMP: loop-mediated isothermal amplification, QBC: quantitative buffy coat

5 ¹ Based on a prevalence of malaria of 0.07

6 ² Based exclusively on infections whose species were identified with PCR (n=70)

7 ³ Only the predominant species was identified in co-infections (n=2)

8 ⁴ TnS misdiagnoses (n=2) + false positive *vivax*-LDH on RDT (n=3)

9 ⁵ Undetected *Plasmodium* infection with corresponding strategy

1
2
3

Table 4: Performance of the *Alethia (ex-illumigene)* LAMP assay

Reference	Area of the study ^a	Gold standard method	Type of study ^b	<i>Alethia</i> LAMP assay ^c	Total patients (n)	Malaria positive (n)	Se	Sp	VPP	VPN
Lucchi <i>et al.</i> 2016 (27)	E	PCR	Retro / Prosp	M	209	144	97.2	93.8	-	-
				MP	209	144	97.2	87.7	-	-
Rypien <i>et al.</i> 2017 (28)	NE	Microscopy	Retro	M	139	75	97.3	93.8	45.2 ^d	99.8 ^d
				MP	132	73	100	91.5	38.2 ^d	100 ^d
Ponce <i>et al.</i> 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 Koninck <i>et al.</i> 2017 (24)	NE	Microscopy / RDT / PCR	Retro	M	108	74	100	100	-	-
Frickmann <i>et al.</i> 2018 (25)	NE	Microscopy / PCR	Prosp	M	1000	238	98.7	99.6	98.7	99.6
Cheaveau <i>et al.</i> 2018 (26)	NE	Microscopy / PCR	Prosp	M	298	25	100	100	100 ^e	100 ^e
Charpentier <i>et al.</i> [this report]	NE	PCR	Retro	M	100	73	97.3	100	-	-
			Prosp	M	331	73	97.3	99.6	94.8 ^f	99.7 ^f

^aE: Malaria endemic area; NE: Malaria non-endemic area

^bRetro: Retrospective study (frozen samples); Prosp: Prospective study (fresh samples)

^cM: Meridian *Alethia* malaria; MP: Meridian *Alethia* malaria Plus

^dBased on a prevalence of malaria of 0.05

^eBased on a prevalence of malaria of 0.044

^fBased on a prevalence of malaria of 0.07

4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

1 REFERENCES

- 2
3
4 1. McCarthy AE, Morgan C, Prematunge C, Geduld J. 2015. Severe malaria in Canada,
5 2001-2013. *Malar J* 14:151.
- 6 2. Hänscheid T. 2003. Current strategies to avoid misdiagnosis of malaria. *Clin Microbiol*
7 *Infect* 9:497–504.
- 8 3. European Centre for Disease Prevention and Control (ECDC). 2015. Surveillance
9 Report, Annual Epidemiological Report for 2015, Malaria.
- 10 4. Tatem AJ, Jia P, Ordanovich D, Falkner M, Huang Z, Howes R, Hay SI, Gething PW,
11 Smith DL. 2017. The geography of imported malaria to non-endemic countries: a meta-
12 analysis of nationally reported statistics. *Lancet Infect Dis* 17:98–107.
- 13 5. Abanyie FA, Arguin PM, Gutman J. 2011. State of malaria diagnostic testing at clinical
14 laboratories in the United States, 2010: a nationwide survey. *Malar J* 10:340.
- 15 6. Agence nationale de sécurité du médicament et des produits de santé (ANSM). 2015.
16 Annales du contrôle national de qualité des analyses de biologie médicale. Parasitologie
17 14PAR1, octobre 2014. Enquête : recensement des cas de paludisme en France
18 métropolitaine au cours de l'année 2013.
- 19 7. Zimmerman PA, Howes RE. 2015. Malaria diagnosis for malaria elimination. *Curr Opin*
20 *Infect Dis* 28:446–454.
- 21 8. World Health Organization (WHO). 2014. Policy brief on malaria diagnostics in low-
22 transmission settings.

- 1 9. Maltha J, Gillet P, Jacobs J. 2013. Malaria rapid diagnostic tests in travel medicine. Clin
2 Microbiol Infect 19:408–415.
- 3 10. World Health Organization (WHO). 2018. Malaria Rapid Diagnostic Test performance.
4 Results of WHO product testing of malaria RDTs: round 8 (2016–2018).
- 5 11. Roth JM, Korevaar DA, Leeflang MMG, Mens PF. 2016. Molecular malaria diagnostics:
6 A systematic review and meta-analysis. Crit Rev Clin Lab Sci 53:87–105.
- 7 12. Hofmann N, Mwingira F, Shekalaghe S, Robinson LJ, Mueller I, Felger I. 2015. Ultra-
8 sensitive detection of *Plasmodium falciparum* by amplification of multi-copy
9 subtelomeric targets. PLoS Med 12:e1001788.
- 10 13. Oriero EC, Jacobs J, Van Geertruyden J-P, Nwakanma D, D’Alessandro U. 2015.
11 Molecular-based isothermal tests for field diagnosis of malaria and their potential
12 contribution to malaria elimination. J Antimicrob Chemother 70:2–13.
- 13 14. Polley SD, González IJ, Mohamed D, Daly R, Bowers K, Watson J, Mewse E,
14 Armstrong M, Gray C, Perkins MD, Bell D, Kanda H, Tomita N, Kubota Y, Mori Y,
15 Chiodini PL, Sutherland CJ. 2013. Clinical evaluation of a loop-mediated amplification
16 kit for diagnosis of imported malaria. J Infect Dis 208:637–644.
- 17 15. Lucchi NW, Demas A, Narayanan J, Sumari D, Kabanywanyi A, Kachur SP, Barnwell
18 JW, Udhayakumar V. 2010. Real-time fluorescence loop mediated isothermal
19 amplification for the diagnosis of malaria. PLoS ONE 5:e13733.
- 20 16. Code de la Santé Publique. 2017. Décret n° 2017-884 du 9 mai 2017 modifiant certaines
21 dispositions réglementaires relatives aux recherches impliquant la personne humaine.
22 Article R. 1121-1-1.

- 1 17. World Health Organization (WHO). 2010. Basic malaria microscopy – Part I: Learner’s
2 guide. Second edition.
- 3 18. Training in Tropical Diseases (TDR) / World Health Organization (WHO). 2015.
4 Microscopy for the detection, identification and quantification of malaria parasites on
5 stained thick and thin blood films in research settings.
- 6 19. Fabre R, Berry A, Morassin B, Magnaval JF. 2004. Comparative assessment of
7 conventional PCR with multiplex real-time PCR using SYBR Green I detection for the
8 molecular diagnosis of imported malaria. *Parasitology* 128:15–21.
- 9 20. Centre national de référence du Paludisme (France). 2017. Rapport annuel d’activité
10 2017, Année d’exercice 2016.
- 11 21. Haute Autorité de Santé (HAS). 2016. Évaluation des actes de diagnostic biologique des
12 infections à *Plasmodium*.
- 13 22. World Health Organization (WHO). 2017. A framework for malaria elimination.
- 14 23. Iglesias N, Subirats M, Trevisi P, Ramírez-Olivencia G, Castán P, Puente S, Toro C.
15 2014. Performance of a new gelled nested PCR test for the diagnosis of imported
16 malaria: comparison with microscopy, rapid diagnostic test, and real-time PCR. *Parasitol*
17 *Res* 113:2587–2591.
- 18 24. De Koninck A-S, Cnops L, Hofmans M, Jacobs J, Van den Bossche D, Philippé J. 2017.
19 Diagnostic performance of the loop-mediated isothermal amplification (LAMP) based
20 illumigene® malaria assay in a non-endemic region. *Malar J* 16:418.
- 21 25. Frickmann H, Hinz R, Rojak S, Bonow I, Ruben S, Wegner C, Zielke I, Hagen RM,
22 Tannich E. 2018. Evaluation of automated loop-mediated amplification (LAMP) for

- 1 routine malaria detection in blood samples of German travelers - A cross-sectional study.
2 Travel Med Infect Dis 24:25–30.
- 3 26. Cheaveau J, Nguyen H, Chow B, Marasinghe D, Mohon AN, Yuan H, Viana G, van
4 Schalkwyk D, Church D, Chan W, Pillai DR. 2018. Clinical Validation of a Commercial
5 LAMP Test for Ruling out Malaria in Returning Travelers: A Prospective Diagnostic
6 Trial. Open Forum Infect Dis 5:ofy260.
- 7 27. Lucchi NW, Gaye M, Diallo MA, Goldman IF, Ljolje D, Deme AB, Badiane A, Ndiaye
8 YD, Barnwell JW, Udhayakumar V, Ndiaye D. 2016. Evaluation of the Illumigene
9 Malaria LAMP: A Robust Molecular Diagnostic Tool for Malaria Parasites. Sci Rep
10 6:36808.
- 11 28. Rypien C, Chow B, Chan WW, Church DL, Pillai DR. 2017. Detection of *Plasmodium*
12 Infection by the illumigene Malaria Assay Compared to Reference Microscopy and
13 Real-Time PCR. J Clin Microbiol 55:3037–3045.
- 14 29. Ponce C, Kaczorowski F, Perpoint T, Miailhes P, Sigal A, Javouhey E, Gillet Y, Jacquin
15 L, Douplat M, Tazarourte K, Potinet V, Simon B, Lavoignat A, Bonnot G, Sow F,
16 Bienvenu A-L, Picot S. 2017. Diagnostic accuracy of loop-mediated isothermal
17 amplification (LAMP) for screening patients with imported malaria in a non-endemic
18 setting. Parasite 24:53.
- 19 30. Groupe recommandations de la Société de Pathologie Infectieuse de Langue Française
20 (SPILF). 2017. Prise en charge et prévention du paludisme d'importation. Mise à jour
21 2017 des RPC 2007.