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Systematic review and meta-analysis of diagnostic accuracy of loop-mediated isothermal amplification (LAMP) methods compared with microscopy, polymerase chain reaction and rapid diagnostic tests for malaria diagnosis

Stephane Picot[a,b], Michel Cucherat[c], Anne-Lise Bienvenu[a,d,e,*]

[a] Malaria Research Unit, SMITh, ICBMS UMR 5246 CNRS-INSA-CPE-Université de Lyon, Campus Lyon-Tech La Doua, 69622 Villeurbanne Cedex, France
[b] Institut de Parasitologie et Mycologie Médicale, Hôpital de La Croix-Rousse, Hospices Civils de Lyon, Lyon, France
[c] Service de Pharmacotoxicologie, Hospices Civils de Lyon, Laboratoire de Biométrie et Biologie Évolutive, CNRS, UMR5558, Université Lyon 1, Lyon, France
[d] Service Pharmacie, Groupement Hospitalier Nord, Hospices Civils de Lyon, France
[e] Service d'Hématologie, Groupement Hospitalier Nord, Hospices Civils de Lyon, France

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ABSTRACT

Background: Diagnosis is a challenging issue for eliminating malaria. Loop-mediated isothermal amplification (LAMP) could be an alternative to conventional methods. This study aimed to evaluate the diagnostic accuracy of LAMP for malaria compared with microscopy, polymerase chain reaction (PCR) and rapid diagnostic tests (RDTs).
Methods and design: MEDLINE, Web of Science and Scopus were searched from inception to 1 July 2019. Prospective and retrospective, randomised and non-randomised, mono-center and multi-center studies, including symptomatic or asymptomatic patients, that reported one LAMP method and one comparator (microscopy, RDT or PCR) were included. PROSPERO registration number: CRD42017075186.
Results: Sixty-six studies published between 2006 and 2019 were included, leading to the analysis of 30,641 LAMP tests. The pooled sensitivity of LAMP remained between 96% and 98%, whichever the comparator. The pooled specificity of LAMP was around 95%, but was a little higher if the best PCR studies were considered. The AUC was found to be >0.98, whichever the subgroup of studies was considered. Diagnostic odds ratio (DOR) was found to be around 1000 for all subgroups, except for *Plasmodium vivax*.
Conclusion: This meta-analysis confirmed that the LAMP method is robust for diagnosing malaria, both in symptomatic and asymptomatic people. Thus, the impact of LAMP for controlling malaria is expected to be important.

Introduction

Eliminating malaria globally appears to be more difficult to achieve than expected (WHO, 2018). Diagnosing malaria requires highly sensitive, reliable and easy-to-perform methods (Landier et al., 2016). Malaria diagnosis can be conducted using microscopy, rapid diagnosis tests (RDT), polymerase chain reaction (PCR), or a combination of these methods. Microscopic examination of Giemsa-stained thick or thin smears is the reference standard, providing quality can be maintained and good expertise is available (Billo et al., 2013). However, there is a lack of expert malaria microscopists, leading to impaired microscopy-based diagnosis, especially for non-*falciparum* species (Ruas et al., 2017). Limit of detection (LOD) may substantially vary, according to the microscopists' experience and training, with a range of 5–100 parasites/μL (Zimmerman and Howes, 2015).

RDTs for malaria (Odaga et al., 2014) have increased the availability of reliable diagnosis in remote areas (Wilson, 2013). RDTs are widely used in endemic areas, with a positive effect on the management of fever cases, but they have definitive limitations. While the sensitivity and specificity of the majority of RDTs are good for *Plasmodium falciparum* (*P. falciparum*), this is not the case for other human malaria species. The LOD of RDTs is too high (100–200 parasites/μL, depending on the test, with the exception of

* Corresponding author at: Malaria Research Unit, SMITh, ICBMS UMR 5246 CNRS-INSA-CPE-Université de Lyon, Campus Lyon-Tech La Doua, 69622 Villeurbanne Cedex, France.
E-mail address: anne-lise.bienvenu@chu-lyon.fr (A.-L. Bienvenu).

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recent ultrasensitive RDTs) to be useful in detecting asymptomatic carriers in cases of gametocyte carriage, which are the main reservoir of *Plasmodium* parasites responsible for continuing transmission. Most RDTs have been designed for detecting HRP2, leading to an emerging selection of parasite clones deleted in HRP2/3 proteins in South America and more recently in Sub-Saharan Africa, with a risk of false-negative results (Menegon et al., 2017).

In this context, molecular detection of malaria would be extremely useful in overcoming these limitations. The molecular detection of malaria parasites using PCR or Q-PCR has indeed proven its capacity in detecting low parasitaemia with a LOD of $\leq$0.05 parasites/μL (Roth et al., 2016). A wide range of PCR methods has been developed for diagnosing malaria, providing valuable information on the parasite biology and pyrogenic threshold. Authors have tried to simplify the method to enable large-scale use in malaria endemic areas, but it should be considered that these methods are unavailable for point-of-care diagnosis without subsequent training and equipment (Amir et al., 2018). PCR is still limited to well-equipped centers that are mostly far from remote endemic areas, which is not ideal for quick commencement of treatment for malaria cases.

Loop-mediated isothermal amplification (LAMP) of DNA was first described in 2000 (Notomi et al., 2000) and its first use for diagnosing malaria was in 2006 (Poon et al., 2006). LAMP is an isothermal molecular method using a DNA polymerase from *Bacillus stearothermophilus*, which has strand displacement activity leading to DNA auto-cycling without temperature changes. The different tests that are available mostly target the mitochondrial genome of the *Plasmodium* parasite. This method of DNA amplification is easier and cheaper to perform than PCR as it requires less equipment and laboratory facilities (Lucchi et al., 2018). It is expected to have a higher sensitivity than microscopy and RDT, and an excellent likelihood of negative value (Ponce et al., 2017). Recent technical developments have led to LAMP being considered as a suitable tool for diagnosis in endemic and non-endemic areas (Polley et al., 2013). Among the questions that remain are the place of the LAMP method in a diagnosis process according to available resources, malaria transmission level, lab equipment and staff training.

This systematic review aimed to evaluate the diagnostic accuracy of LAMP methods compared with microscopy, PCR and RDTs for malaria diagnosis in symptomatic and asymptomatic patients from endemic and non-endemic areas. The following questions were asked: (1) When compared with the microscopic examination of blood smears or RDTs (reference standards) and PCR (standard of truth) what are the performances (sensitivity, specificity, positive predictive value, negative predictive value) of LAMP methods (index method) for detecting human *Plasmodium* parasites? (2) Is there a difference among main *Plasmodium* species when LAMP tests are used for detection? (3) Is there a difference in LAMP test performances among symptomatic and non-symptomatic patients? (4) Is there a difference in LAMP test performance among the available methods?

Methods

This systematic review and meta-analysis followed the Preferred Reporting Items for Systematic review and Meta-Analyses (PRISMA) guidelines and the *Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy* (McGrath et al., 2017). The study protocol was registered with the international Prospective Register of Systematic reviews (PROSPERO) on 24 August 2017 and was last updated on 25 September 2017 (registration number CRD42017075186).

Search strategy

Literature search strategies were developed using medical subject headings (MESH) in the National Library of Medicine and text words related to malaria diagnosis and LAMP. MEDLINE, (("Lamp"[Journal] OR "lamp"[All Fields]) AND ("malaria"[MeSH Terms] OR "malaria"[All Fields])) Web of Science and Scopus were searched from inception to 1 July 2019. To ensure literature saturation, the reference list of included and excluded studies was scanned.

Eligibility criteria

Prospective and retrospective, randomised and non-randomised, mono-center and multi-center studies, from symptomatic or asymptomatic patients without age limitation who reported at least one LAMP method for malaria diagnosis (*Plasmodium* genus or species) and one comparator (microscopy, RDT or PCR), were included up to July 2019. Studies from malaria endemic or non-endemic areas, and from research, hospital and tertiary care settings were included. If a 2×2 table of true positive, false negative, true negative, and false positive counts was unavailable, data were extracted from the original article. There was no language limitation for inclusion. Case reports, animal (including mosquitoes) studies, studies using parasite clones from culture or parasite DNA from collection and methodological studies without clinical samples were excluded.

Study selection

After searches in the proposed databases had been completed, a single library was created in Zotero (Version 5.0.18, Corporation for Digital Scholarship, Virginia, USA). The duplicates were removed using Zotero and then manually checked. A Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) was used to manage data and the literature search. Two identical copies of Excel spreadsheets were created and ascribed to two independent reviewers.

The first step was to screen the titles and abstracts of the selected articles to exclude studies according to the exclusion criteria. The reviewers had to justify the exclusion of any items in the spreadsheet. A consensus meeting avoided any discrepancy between the two spreadsheets. Studies were included for the next step if there was any uncertainty. Based on the first selection list, the next step was to read the full texts to exclude studies that did not meet inclusion criteria. This second step was validated by another consensus meeting to select which article would be considered eligible for review. Disagreements were resolved by discussion and reasons for excluding studies were recorded. A data extraction form was built to compile data from the included studies.

Data extraction and checking

Variables were recorded such as: title, references, authors, country of the study (endemic vs non-endemic), participants (symptomatic vs non-symptomatic), interventions (genus-specific and/or species-specific detection, commercial or in-house method, prospective or retrospective study), comparators (PCR, microscopy and RDT), and number of participants. To ensure reproducibility and completeness of data extraction, a predesigned Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) compiling all variables to be extracted was used. Duplicate, overlapping or companion studies that were detected during the data extraction process were excluded. Two investigators (ALB, SP) independently collected the true positive, false positive, true negative, and false negative counts. Disagreements over data extraction were resolved

by discussion. Data were centrally checked by an independent operator for completeness, plausibility, and integrity, before synthesis.

Quality assessment

The methodological quality of the included studies was evaluated to reduce systematic biases and inferential errors of the extracted data. Risk of bias of the included studies was independently assessed by two reviewers (ALB, SP) using Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) (Whiting et al., 2011). QUADAS-2 assesses four areas of bias: patient selection, index test, reference standards, and flow/timing. A risk of bias summary and graph were generated in Review Manager 5 (RevMan version 5.3.5, http://community.cochrane.org/tools/review-production-tools/revman-5) to provide a synthetic analysis of bias. Moreover, predefined sensitivity analyses after excluding studies judged to have potential risk of bias were performed. Studies without high risk of bias for any of the defined parameters were considered for the 'best studies' subgroup.

Data synthesis

The software used for meta-analysis was RevMan 5. Values of test accuracy were compared with microscopic examination of blood smears and non-isothermal PCR methods – including sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and 95% confidence intervals – using data extracted from sources (true positive, true negative, false positive, and false negative) or calculated from the available data. Given the general use of microscopic examination of thin or thick blood smears as reference standard, microscopy was considered as the clinical practice comparator. PCR was the most relevant to compare with LAMP; thus, PCR was considered as the direct comparator. RDTs are widely used in endemic malaria areas, but not frequently used as a comparator for sensitive diagnostic tests; thus, RDTs were considered as a second clinical practice comparator. Results of individual study were graphically presented on forest plots, as well as on receiver operating characteristic (ROC) curves. To evaluate overall performances of the LAMP tests, a bivariate (bivariate diagnostic random effect meta-analysis) and univariate analyses

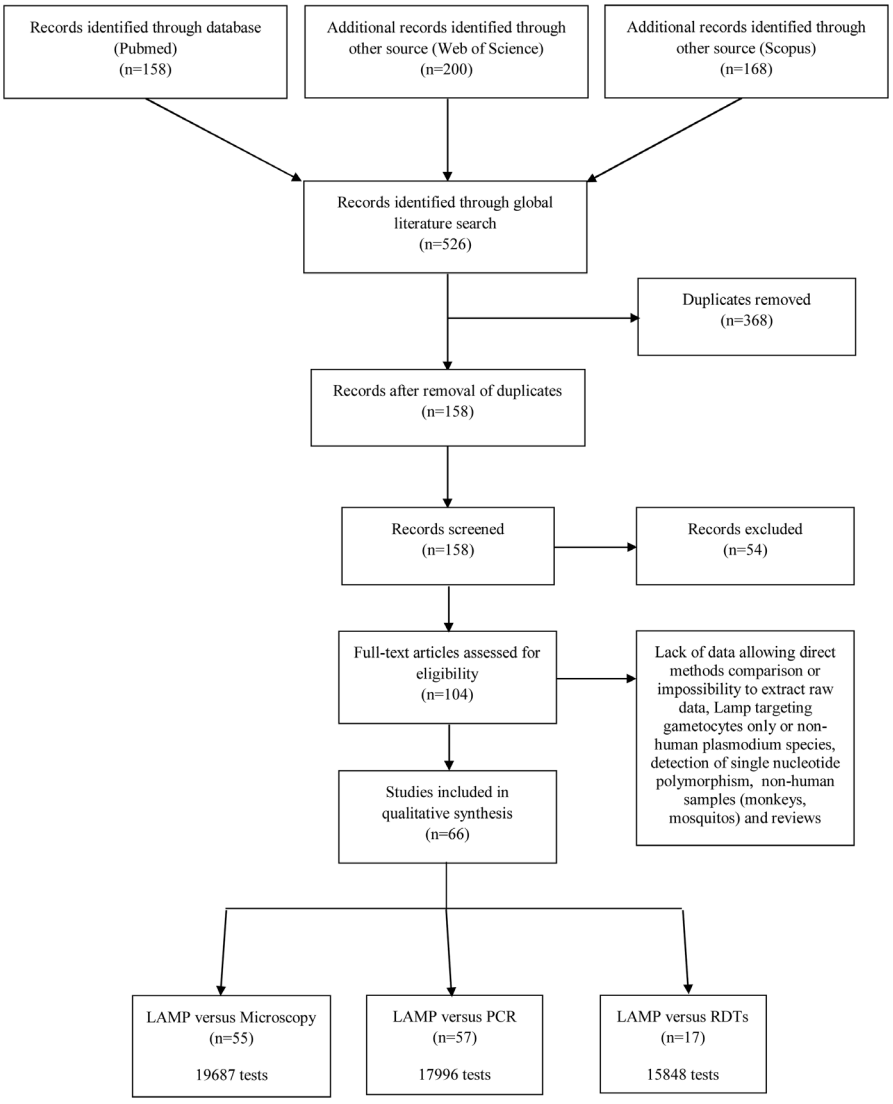

Figure 1. Flow chart. Abbreviations: LAMP, loop-mediated isothermal amplification; PCR, polymerase chain reaction; RDT, rapid diagnostic tests.

(DerSimonian and Laird random effect model) were conducted using R 3.5.2 software (R Foundation for Statistical Computing, Vienna, Austria). Summary point estimates of the pairs of sensitivity and specificity with their 95% confidence interval, diagnostic odd ratios with their 95% confidence interval, and receiver operating characteristic (SROC) curves were obtained.

Results

Search results

The literature search identified 526 records from three different sources: 158 from MEDLINE, 168 from SCOPUS and 200 from Web of sciences (Figure 1). After removal of duplicates, the remaining studies ($n = 158$) were screened. Fifty-four records were excluded, as they did not meet the inclusion criteria. The 104 eligible studies were assessed in detail. Sixty-six studies were included (Tables 1–3). The reasons for exclusion of the 38 studies were: lack of data allowing direct method comparison or impossibility of data extraction, LAMP targeting gametocytes only or non-human plasmodium species (*Plasmodium chabaudi*), detection of single-nucleotide polymorphism, non-human samples (monkeys, mosquitos) and reviews.

Study characteristics

Sixty-six studies published between 2006 and 2019 were included, leading to 30,641 individual LAMP tests compared either with microscopy, RDT or PCR, or a combination of these comparators. Thirty-four of 66 (51%) studies were retrospective and seven (10.6%) used samples from asymptomatic people.

Table 1
List of included studies comparing LAMP with microscopy.

	Years	Authors	TP	FP	FN	TN
1	2014	Aydin-Schmidt et al. (2014)	115	1	0	749
2	2016	Britton et al. (2016b)	59	3	39	44
3	2018	Cheaveau et al. (2018)	51	7	1	290
4	2010	Chen et al. (2010)	115	0	2	30
5	2015	Cuadros et al. (2015)	0	11	0	151
6	2017	De Koninck et al. (2017)	11	1	0	18
7	2014	Dinzouna-Boutamba et al. (2014)	96	41	0	40
8	2018	Frickmann et al. (2018)	235	3	3	759
9	2014	Ghayour Najafabadi et al. (2014)	68	0	0	40
10	2018	Girma et al. (2018)	118	7	0	437
11	2007	Han et al. (2007)	67	3	1	50
12	2019	Hartmeyer et al. (2019)	18	9	0	11
13	2018	Kaur et al. (2018)	145	3	0	17
14	2018	Kollenda et al. (2018)	177	57	0	289
15	2019	Kudyba et al. (2019)	33	6	0	52
16	2011	Lau et al. (2011)	13	0	2	59
17	2016	Lau et al. (2016)	132	0	0	69
18	2012	Lee et al. (2012)	46	19	1	62
19	2012	Lu et al. (2012)	160	0	4	50
20	2010	Lucchi et al. (2010)	89	1	5	11
21	2016	Lucchi et al. (2016)	135	9	1	64
22	2015	Marti et al. (2015)	43	4	0	158
23	2018	McCreesh et al. (2018)	16	27	15	1861
24	2014	Mohon et al. (2014)	105	1	1	104
25	2016	Mohon et al. (2016)	69	4	0	67
26	2015	Morris et al. (2015)	19	46	0	,918
27	2016	Ocker et al. (2016)	116	0	2	15
28	2015	Oriero et al. (2015a)	104	23	0	214
29	2014	Patel et al. (2014)	91	0	5	45
30	2017	Piera et al. (2017)	73	0	0	19
31	2010	Polley et al. (2010)	28	0	2	113
32	2013	Polley et al. (2013)	52	24	0	625
33	2017	Ponce et al. (2017)	79	14	0	206
34	2006	Poon et al. (2006)	96	1	6	99
35	2010	Pöschl et al. (2010)	71	2	0	32
36	2017	Rypien et al. (2017)	76	4	0	60
37	2014	Sattabongkot et al. (2014)	177	15	22	466
38	2018	Sattabongkot et al. (2018)	6	88	0	3559
39	2015	Sema et al. (2015)	30	8	0	44
40	2017	Serra-Casas et al. (2017)	52	203	0	912
41	2013	Singh et al. (2013)	68	4	1	16
42	2017	Singh et al. (2017)	248	0	9	60
43	2011	Sirichaisinthop et al. (2011)	59	0	1	50
44	2013	Surabattula et al. (2013)	162	1	6	14
45	2018	Tambo et al. (2018)	23	24	0	2593
46	2011	Tao et al. (2011)	59	0	1	29
47	2017	Tegegne et al. (2017)	10	5	0	72
48	2015	Vallejo et al. (2015)	80	14	0	184
49	2018	Vásquez et al. (2018)	31	8	0	492
50	2018	Viana et al. (2018)	226	13	0	761
51	2018	Vincent et al. (2018)	62	2	0	51
52	2009	Yamamura et al. (2009)	85	1	6	2
53	2018	Zelman et al. (2018)	2	5	1	1487

Abbreviations: TP, true positive; FP, false positive; FN, false negative; TN, true negative; LAMP, loop-mediated isothermal amplification.

Table 2
List of included studies comparing LAMP with PCR.

	Years	Authors	TP	FP	FN	TN
1	2014	Aydin-Schmidt et al. (2014)	105	2	10	748
2	2017	Aydin-Schmidt et al. (2017)	20	2	29	2957
3	2016	Britton et al. (2016a)	62	3	36	44
4	2018	Cheaveau et al. (2018)	57	0	0	291
5	2010	Chen et al. (2010)	116	0	1	30
6	2015	Cook et al. (2015)	10	3	0	984
7	2017	Cuadros et al. (2017)	29	11	0	387
8	2014	Dinzouna-Boutamba et al. (2014)	128	9	0	40
9	2014	Ghayour Najafabadi et al. (2014)	68	2	0	40
10	2018	Girma et al. (2018)	121	4	0	562
11	2007	Han et al. (2007)	67	3	1	50
12	2019	Hartmeyer et al. (2019)	27	0	1	10
13	2017	Hayashida et al. (2017)	27	0	3	31
14	2013	Hopkins et al. (2013)	178	3	21	70
15	2018	Kaur et al. (2018)	148	0	0	17
16	2018	Kollenda et al. (2018)	234	0	9	280
17	2019	Kudyba et al. (2019)	39	0	1	51
18	2011	Lau et al. (2011)	12	1	0	61
19	2016	Lau et al. (2016)	132	2	0	67
20	2012	Lee et al. (2012)	64	1	0	63
21	2012	Lu et al. (2012)	160	0	2	50
22	2010	Lucchi et al. (2010)	89	1	1	14
23	2016	Lucchi et al. (2016)	140	8	4	57
24	2015	Marti et al. (2015)	43	0	0	158
25	2014	Mohon et al. (2014)	106	0	2	103
26	2016	Mohon et al. (2016)	71	2	0	67
27	2016	Ocker et al. (2016)	116	0	1	15
28	2015	Oriero et al. (2015a)	100	5	7	72
29	2015	Oriero et al. (2015b)	120	7	11	203
30	2013	Patel et al. (2013)	70	0	4	46
31	2014	Patel et al. (2014)	91	0	5	45
32	2017	Perera et al. (2017)	67	0	2	630
33	2017	Piera et al. (2017)	73	0	0	19
34	2010	Polley et al. (2010)	28	0	2	113
35	2013	Polley et al. (2013)	62	14	1	624
36	2017	Ponce et al. (2017)	85	4	0	210
37	2006	Poon et al. (2006)	96	1	5	100
38	2010	Pöschl et al. (2010)	72	0	1	32
39	2017	Rypien et al. (2017)	78	2	0	60
40	2014	Sattabongkot et al. (2014)	67	0	5	147
41	2018	Sattabongkot et al. (2018)	88	0	0	88
42	2015	Sema et al. (2015)	31	7	0	44
43	2017	Serra-Casas et al. (2017)	235	20	21	227
44	2013	Singh et al. (2013)	72	0	1	16
45	2017	Singh et al. (2017)	248	0	11	58
46	2011	Tao et al. (2011)	57	2	0	30
47	2017	Tegegne et al. (2017)	10	5	0	72
48	2015	Vallejo et al. (2015)	64	17	6	191
49	2018	Vásquez et al. (2018)	39	0	0	492
50	2018	Viana et al. (2018)	220	19	0	761
51	2018	Vincent et al. (2018)	64	0	2	51
52	2016	Xu et al. (2016)	59	0	10	11
53	2009	Yamamura et al. (2009)	86	0	2	2

Abbreviations: TP, true positive; FP, false positive; FN, false negative; TN, true negative; LAMP, loop-mediated isothermal amplification; PCR, polymerase chain reaction.

Table 3
List of included studies comparing LAMP with RDTs.

	Years	Authors	TP	FP	FN	TN
1	2014	Aydin-Schmidt et al. (2014) (Asymptomatic)	13	37	0	415
2	2014	Aydin-Schmidt et al. (2014) (Suspected)	115	6	0	744
3	2017	Aydin-Schmidt et al. (2017)	13	9	0	2997
4	2015	Cook et al. (2015)	10	8	1	978
5	2015	Cuadros et al. (2015)	3	8	0	159
6	2017	De Koninck et al. (2017)	8	4	0	18
7	2014	Dinzouna-Boutamba et al. (2014)	137	0	0	40
8	2018	Girma et al. (2018)	20	105	0	437
9	2019	Hartmeyer et al. (2019)	18	9	0	10
10	2018	Kaur et al. (2018)	143	5	0	17
11	2018	McCreesh et al. (2018)	16	27	15	1861
12	2016	Mohon et al. (2016)	61	12	0	79
13	2015	Morris et al. (2015)	19	46	0	3918
14	2015	Oriero et al. (2015b)	126	1	0	236
15	2014	Sattabongkot et al. (2014)	65	0	5	147
16	2018	Tambo et al. (2018)	23	24	0	2593
17	2017	Tegegne et al. (2017)	9	6	0	72

Abbreviations: TP, true positive; FP, false positive; FN, false negative; TN, true negative; LAMP, loop-mediated isothermal amplification; RDT, rapid diagnostic tests.

Twenty-seven (41%) were conducted in South-East Asian countries, 18 (27%) in Africa, 16 (24%) in Europe or North America from malaria imported cases, and five (7%) in South America. The reference methods used for comparison with LAMP were microscopy for 53 studies (19,418 tests), PCR for 55 studies (17,479 tests) and RDTs for 17 studies (2065 tests). Most of the LAMP tests (33 of 66) were performed using LoopAmp (Eiken, Tochigi, Japan), 26 (39%) using an in-house method or other commercial test, and seven (10%) using Illumigene Malaria (Meridian Biosciences, Cincinatti, USA). Three methods were used to read LAMP tests averaged among naked-eye, turbidimetry or fluorescence. Among these studies, 11 were designed to detect *P. falciparum*, 13 to detect *Plasmodium vivax* (*P. vivax*) and 10 to detect *P. falciparum* and *P. vivax*. Other studies were designed to detect different combinations of *Plasmodium* species. The vast majority of studies used blood samples, but saliva and urine were used in three studies.

Quality and heterogeneity of diagnostic studies

Risk of bias for patient selection was considered low in 56% of the diagnostic studies and high in 36% (Figure 2). Reporting and execution of LAMP was adequate in 97% of the studies (Figure 2). The quality of verification with a reference standard was good in 100% of the studies (Figure 2). Statistical analysis of heterogeneity found no substantial or moderate heterogeneity between studies included in the analysis of LAMP compared with microscopy and/or PCR, as well as for the sub-group analysis of *P. vivax*. The value of I^2 was 0% according to the bivariate analysis (restricted maximal likelihood, REML) and <60% according to the univariate analysis (diagnostic odds ratio, DOR).

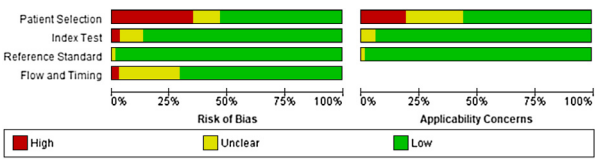

Figure 2. Risk of bias graph of studies included in the meta-analysis.

Table 4
Data synthesis of LAMP results compared with microscopy, and/or PCR or RDT.

	LAMP vs microscopy and PCR	LAMP vs microscopy	LAMP vs microscopy (best studies)	LAMP vs PCR	LAMP vs PCR (best studies)	LAMP vs PCR (*vivax*)	LAMP vs RDT
Bivariate analysis (REML)							
Sensitivity (95% CI lb–ub)	0.977 (0.965–0.985)	0.971 (0.957–0.980)	0.974 (0.960–0.984)	0.971 (0.957–0.980)	0.961 (0.935–0.976)	0.948 (0.800–0.988)	0.966 (0.921–0.986)
Specificity (95% CI lb–ub)	0.947 (0.924–0.964)	0.956 (0.938–0.968)	0.951 (0.927–0.967)	0.956 (0.938–0.968)	0.984 (0.968–0.988)	0.956 (0.865–0.987)	0.964 (0.921–0.984)
AUC	0.985	0.985	0.985	0.985	0.987	0.982	0.984
DOR (2.5–97.5%)	1061.447 (595.199–1892.929)	913.979 (542.306–1540.381)	959.506 (511.141–1801.169)	913.979 (542.306–1540.381)	1658.503 (824.983–3334.165)	462.891 (76.554–2798.903)	911.208 (295.097–2813.651)
I^2 (%)	0	0	0	0	0	0	0
Univariate analysis (DOR)							
OR (95% CI)	1221.186 (695.726–2143.512)	1102.801 (651.748–1866.015)	1158.573 (619.187–2167.826)	1102.801 (651.748–1866.015)	1772.017 (890.516–3526.096)	504.757 (84.814–3003.993)	943.033 (301.015–2954.371)
I^2 (%)	50.0	63.1	58.6	63.1	64.8	83.9	70.7

Abbreviations: LAMP, loop-mediated isothermal amplification; PCR, polymerase chain reaction; REML, restricted maximal likelihood; DOR, diagnostic odds ratio; RDT, rapid diagnostic tests.

Results of synthesis and sensitivity analysis

Table 4 shows the pooled sensitivity and specificity, AUC, DOR and I^2 according to the bivariate analysis (REML) and OR and I^2 according to the univariate analysis (DOR).

LAMP compared with microscopy

Fifty-five studies comparing LAMP with microscopy were included, for a total of 19,687 tests. Most of the studies showed a sensitivity of >0.95 (43 of 55, 78%) and a specificity of >0.95 (33 of 55, 60%) (Figure 3). There was no difference between pooled sensitivity of all included studies (0.971 [0.965–0.985]) compared with a selection of the best studies (0.974 [0.960–0.984]). Few studies showed lower sensitivity or specificity values, mainly due to the use of non-blood samples or *Plasmodium* species-specific tests. Two studies used samples from patients during pregnancy, and six studies tested asymptomatic people. While a decrease in sensitivity and specificity may be expected for pregnant women and asymptomatic people, considering the lower parasitaemia, no specific impact of pregnancy or asymptomatic malaria was detected (Figure 4). There was no evidence of a positive or negative impact of LAMP providers (either Eiken, Meridian, or in-house) (Figure 5) and the reader methods (either naked-eye, turbidimeter or fluorescence) (Figure 6).

LAMP compared with PCR

Fifty-seven studies comparing LAMP with PCR were included, for a total of 17,996 tests. Most of the studies showed a sensitivity of >0.95 (48 of 57, 84%) and a specificity of >0.95 (32 of 57, 56%) (Figure 7). Twenty-eight studies had a prospective design and 29 a retrospective design. Ten of these studies detected *P. vivax* only (17%) and nine detected *P. falciparum* only (15.8%). Most of the other studies were designed to detect two or more *Plasmodium* species. Eight studies (14%) were designed to test asymptomatic people and two (3.5%) to test pregnant women; there was no evidence of an impact of these conditions on LAMP results (Figure 8).

LAMP compared with RDTs

Seventeen studies comparing LAMP with RDTs were included, for a total of 15,848 tests. Most of the samples were collected from asymptomatic volunteers (13,585 tests (68%), seven of 17 studies). The sensitivity was 1.0 in 14 of 17 (82%) and specificity was >0.95 in 10 of 17 studies (59%) (Figure 9). The study designs were

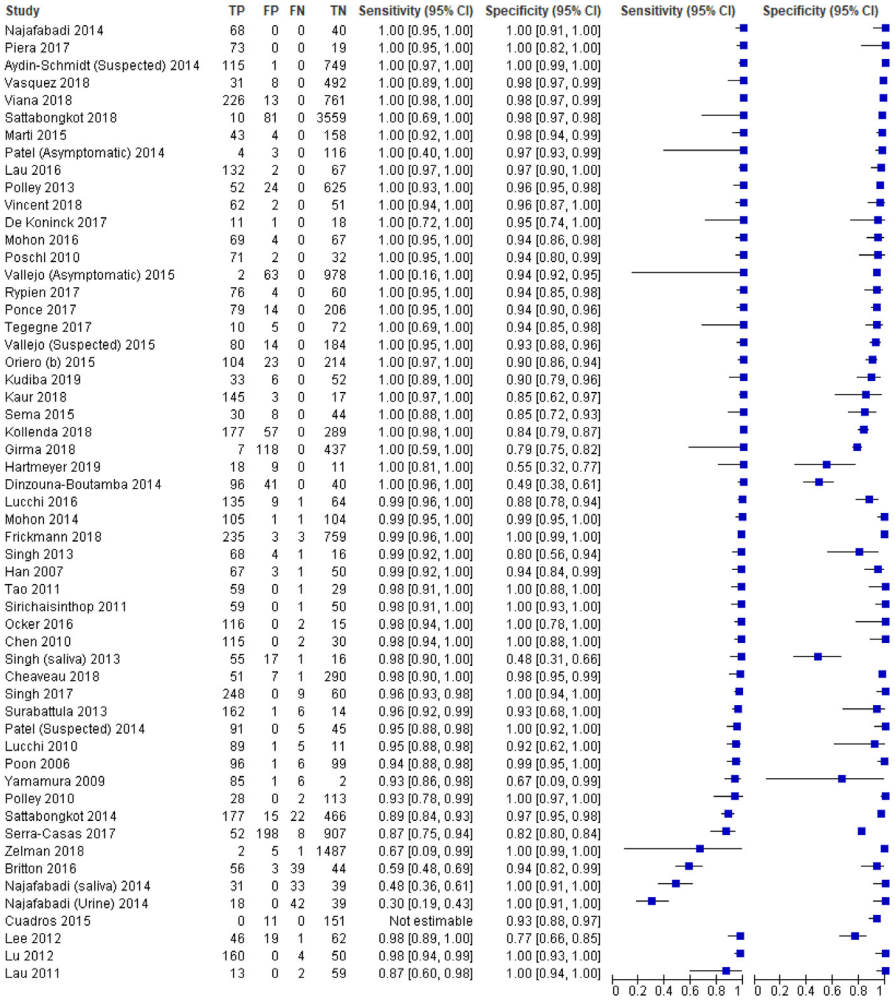

Figure 3. Forest plot of LAMP compared with microscopy. Abbreviations: TP, true positive; FP, false positive; FN, false negative; TN, true negative; LAMP, loop-mediated isothermal amplification.

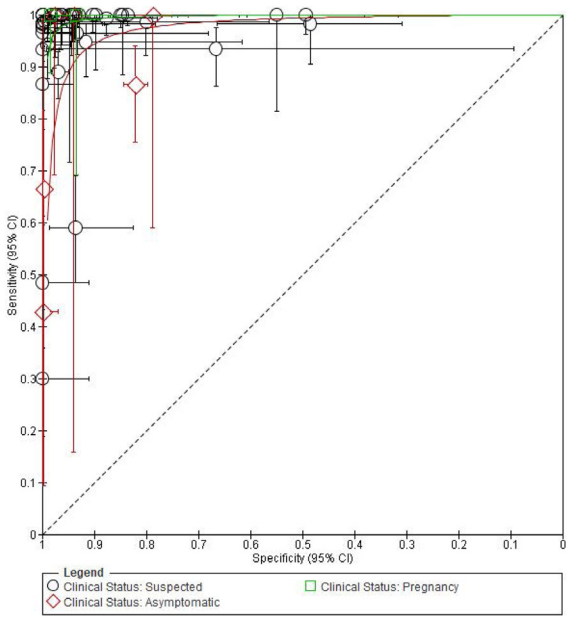

Figure 4. Receiver operating characteristic of LAMP compared with microscopy, according to clinical status of included patients. Abbreviations: LAMP, loop-mediated isothermal amplification.

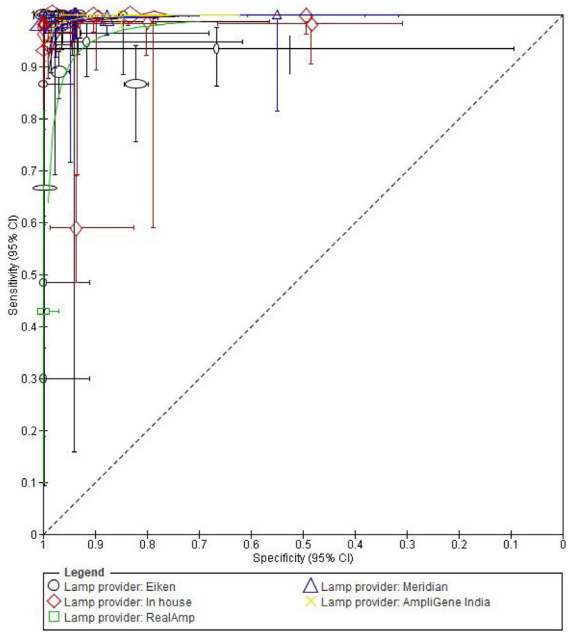

Figure 5. Receiver operating characteristic of LAMP compared with microscopy, according to LAMP test providers. Abbreviations: LAMP, loop-mediated isothermal amplification.

prospective for 13 of 17 studies (76%). The majority of samples were collected from different studies conducted in Zanzibar (9,329 of 15,848, 58.8%) between 2014 and 2017.

Overall performance of LAMP tests

The pooled sensitivity of LAMP remained between 96–98%, whichever the comparator (microscopy PCR or RDT). The pooled specificity of LAMP was around 95%, but a little higher (98%) if best PCR studies were considered. If the analysis was performed for LAMP tests extracted from a selection of best included studies for microscopy, defined as studies presenting the lower risk of bias, the accuracy measures of LAMP were closed to the analysis of the overall population for pooled microscopy and PCR (0.974 [0.960–0.984] compared with 0.977 [0.965–0.985]). The AUC were found to be >0.98, whichever the subgroup of studies considered. For all subgroups of studies, DOR was demonstrated to be around 1000 using a bivariate analysis. Using univariate analysis, OR was >1000 for all subgroups. Interestingly, a higher DOR or OR was observed if LAMP was compared with PCR. If the subgroup *vivax* was considered, DOR and OR were much lower at around 500, confirming the difficulty of *vivax* malaria diagnosis.

Discussion

Strengthening malaria diagnosis should be a priority in both endemic and non-endemic areas (Nema et al., 2019). Malaria elimination is expected by 2030 in many endemic countries, leading to a substantial decrease in a patient's parasitaemia and increase in the proportion of asymptomatic malaria parasite carriers. Microscopy is an appropriate tool when the number of patients suffering with malaria is high enough to allow permanent training and experience of microscopists, whereas RDTs are appropriate when the mean parasitaemia is over the limit of detection of RDT. New accurate diagnosis methods are needed to address the new issues related to changing malaria epidemiology. Among these, high sensitivity is a prerequisite. The LAMP method is probably one of the most interesting recently introduced diagnostic tests, despite its limitation of requiring well-equipped labs with high levels of resources. This method has been tested both in endemic and non-endemic areas in different conditions. The purpose of this study was to compared and analyse the performance of LAMP method versus microscopy, RDTs and PCR.

An extensive search was performed of all the available studies, without limitations in reference methods, study areas and clinical presentation of tested people, including both symptomatic and asymptomatic. The vast majority of these studies were conducted between 2015 and 2019; four studies were conducted before 2010, demonstrating the very recent interest in this diagnostic method. Statistical heterogeneity was tested using the I^2 statistic, which measures the variation across studies due to inter-study heterogeneity. Heterogeneity was expected to be related to the LAMP method (in-house or commercial, species-specific detection or not, DNA target used), the method of test reading (automated, naked-eye), the patients (immune or non-immune, area of contamination, levels of parasitaemia), and the comparators (microscopy, PCR or RDT). Of note, there was no significant heterogeneity among the studies included in the meta-analysis, rendering the results conclusive. Moreover, there was no evidence of significant publication biases according to the QUADAS-2. Applicability of the test accuracy estimates generated in this study was high due to the high number of included studies, leading to approximately 20,000 tests performed for each comparator, the low risk of bias and the absence of heterogeneity of the included studies.

The AUC >0.98 demonstrated that LAMP is a test with an excellent specificity and sensitivity. Whichever the group of studies that was considered, a DOR around 1,000 reflected a test with an excellent discriminant power. Consistent ORs were found using univariate analysis, supporting that the results were robust whichever the method of analysis that was used. A higher DOR or OR was observed if LAMP was compared with PCR, which may be explained by the fact that LAMP and PCR are both molecular tests, whereas microscopy is not; PCR was also considered as the direct

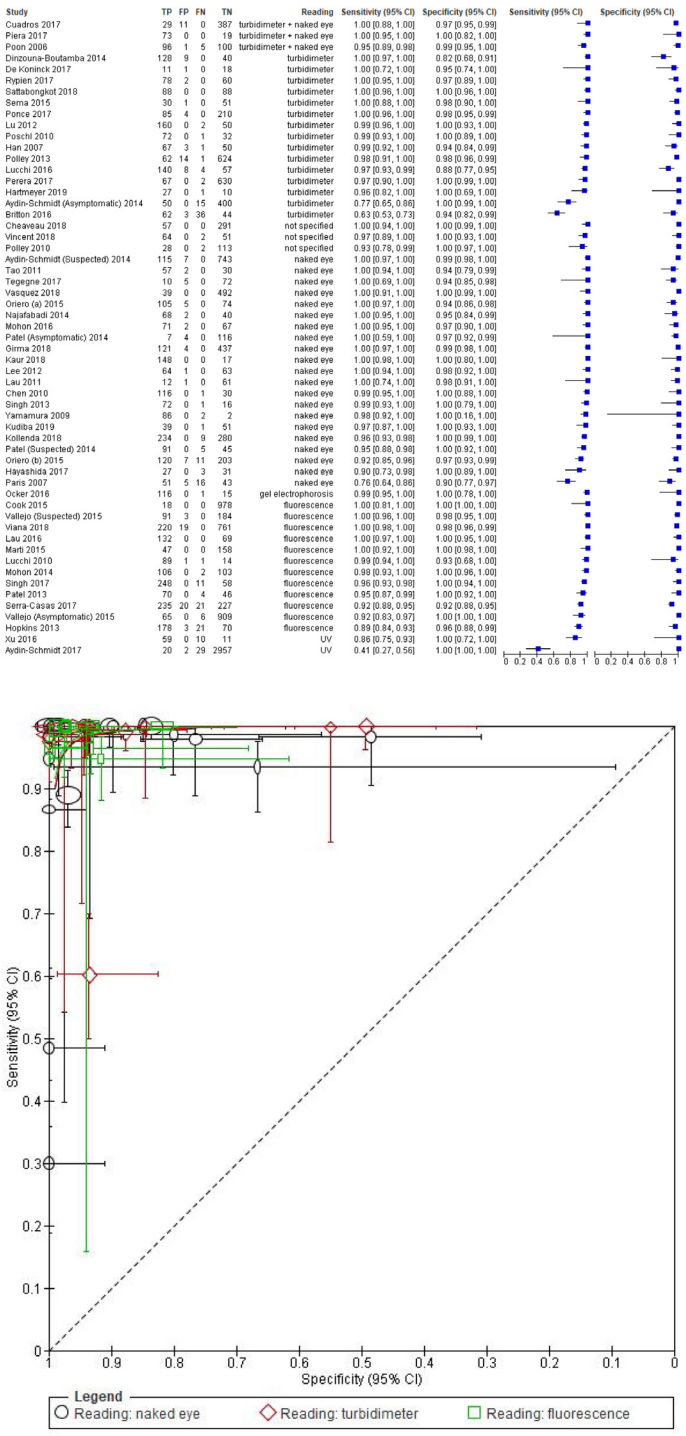

Figure 6. Forest plot and receiver operating characteristic of LAMP, compared with microscopy according to reader method. Abbreviations: TP, true positive; FP, false positive; FN, false negative; TN, true negative; LAMP, loop-mediated isothermal amplification.

comparator in this meta-analysis and microscopy as the clinical practice comparator. If the *vivax* subgroup of studies was considered, DOR and OR were much lower, indicating that the test is a little less efficient in detecting *vivax* malaria. Among the included studies, the LAMP method varied depending on the targeted *Plasmodium* species, primer sequences, amplification time, method for signal detection, commercial or in-house method, and samples that were used (blood, saliva or urine). Despite this, no significant changes in the test performances were observed, except for non-blood samples (saliva and urine) showing lower diagnosis accuracy, as previously demonstrated by biological evidence.

The primary audience of this meta-analysis is clinicians, biologists, public health policymakers, and stakeholders. The results of this study may help them to choose the most appropriate first-line diagnosis method depending on the local requirements

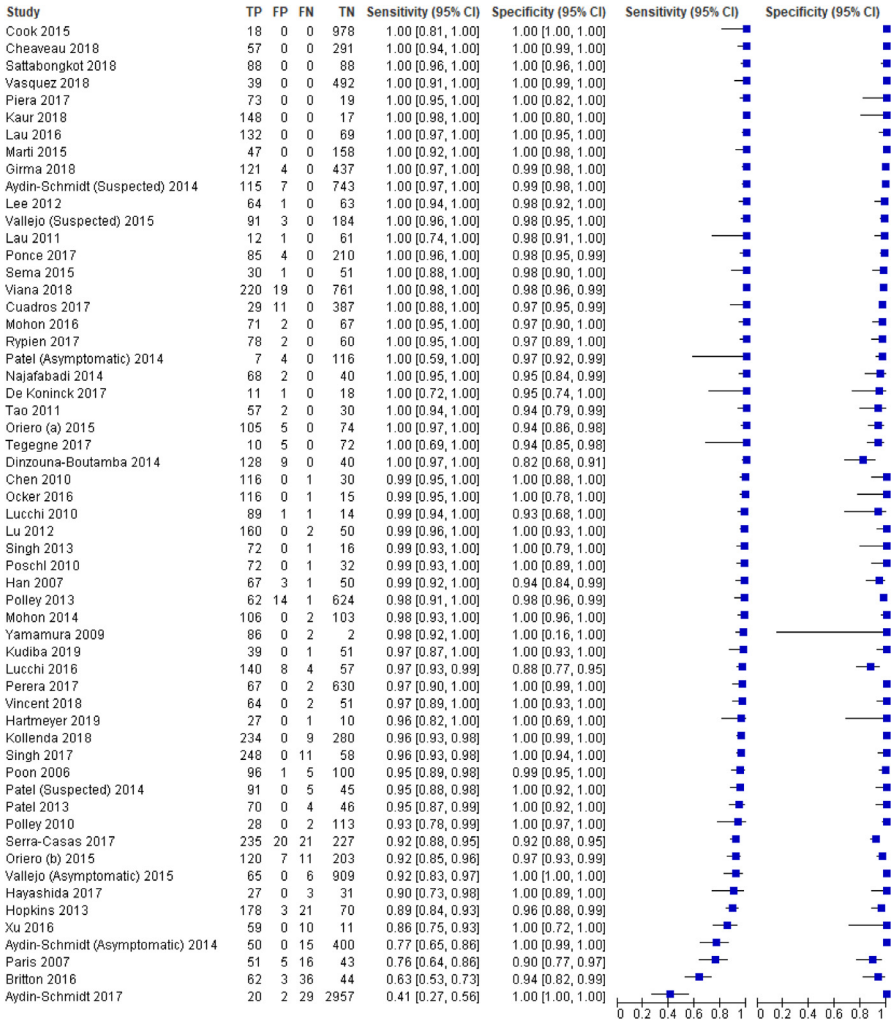

Figure 7. Forest plot of LAMP compared with PCR. Abbreviations: LAMP, loop-mediated isothermal amplification; PCR, polymerase chain reaction.

and availabilities. Microscopic analysis of stained blood smears has been the reference for malaria diagnosis for decades. However, its limitations linked to the required skills and recent availability of new methods may provide an opportunity for changing the malaria diagnosis paradigm. This study gathered evidence for the future of malaria diagnosis and the implementation of LAMP as a reference test for malaria diagnosis.

This meta-analysis had some limitations. First, the search strategy was performed without using search filters, but unpublished data and abstracts from meetings were not taken into account since all the requested information related to the study quality were unavailable. Considering the high number of included studies and the absence of heterogeneity among the included studies, it is expected that potential missing studies should not have a significant impact on the meta-analysis results. Second, studies from endemic and non-endemic areas were included and pooled, leading to the possibility of bias linked to the level of circulating parasitaemia in the studied population. However, the aim of this study was to evaluate the overall diagnosis accuracy of LAMP for malaria and this question was not related to the level of malaria endemicity. The potential impact of the transmission area could be detected by the difference between symptomatic and asymptomatic people. The data analysis showed that there was no difference in diagnosis accuracy of LAMP using blood samples from these two populations. Third, the LAMP method was compared with microscopy, RDTs and PCR. None of these methods should be considered as a gold standard

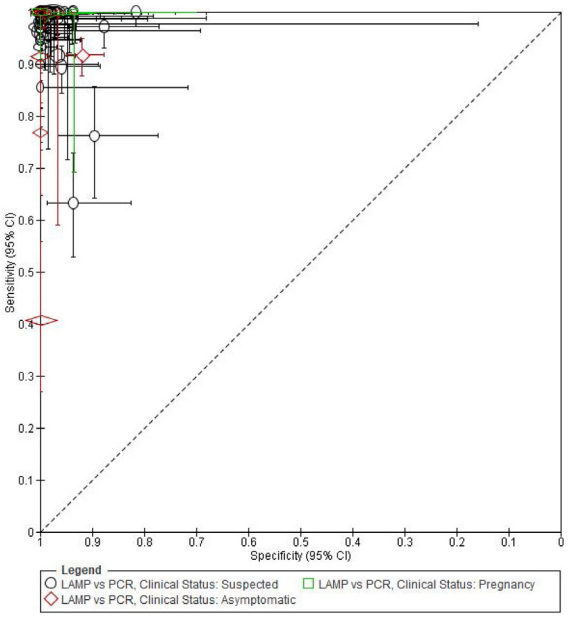

Figure 8. Receiver operating characteristic of LAMP compared with PCR, according to clinical status of included patients. Abbreviations: TP, true positive; FP, false positive; FN, false negative; TN, true negative; LAMP, loop-mediated isothermal amplification; PCR, polymerase chain reaction.

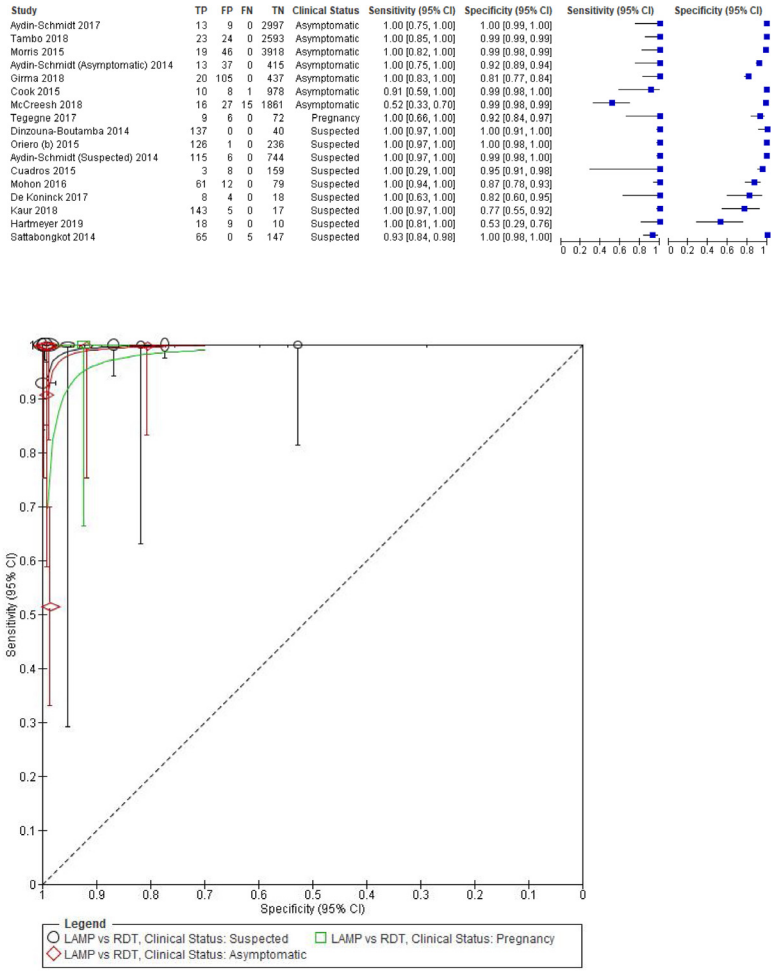

Figure 9. Forest plot and receiver operating characteristic of LAMP versus RDT. Abbreviations: TP, true positive; FP, false positive; FN, false negative; TN, true negative; LAMP, loop-mediated isothermal amplification; RDT, rapid diagnostic tests.

since methodological differences may lead to significant differences in the limit of detection or performances. Moreover, the training and experience of microscope operators have a direct impact on the quality of results. Considering this limitation, PCR is certainly the best comparator for LAMP.

Conclusion

The challenge of diagnosing malaria moving forward is detecting asymptomatic carriers presenting low parasitaemia in endemic areas where transmission is declining. Microscopy and RDTs are highly efficient for diagnosing febrile malaria patients, and PCR shows high sensitivity and specificity while its practicability is low. The position of malaria LAMP in this context needs to be clarified; therefore the first step is to compare its performances with the historical methods for both symptomatic and asymptomatic patients. This meta-analysis clearly confirms a very high DOR (>1000) of LAMP compared with microscopy, RDT and PCR. Malaria LAMP is now widely recommended as a first-line method for diagnosing imported malarial cases in non-endemic countries, due to its high negative predictive value. If the same performances are observed when testing symptomatic and asymptomatic patients, then the impact of LAMP for the control of malaria in the near future is expected to be important.

Authors' contribution

SP and ALB drafted the manuscript and contributed to the development of the selection criteria, the risk of bias assessment strategy and data extraction criteria. MC performed the statistical analysis. ALB developed the search strategy. SP provided expertise on malaria diagnosis and MC expertise on meta-analysis. All authors read, provided feed-back and approved the final manuscript.

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Ethical approval

Not applicable.

Conflict of interest

None.

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