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Rapid diagnostic tests for Plasmodium vivax malaria in endemic countries (Protocol)

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TABLE OF CONTENTS

[Diagnostic Test Accuracy Protocol]

Rapid diagnostic tests for *Plasmodium vivax* malaria in endemic countries

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ABSTRACT

This is a protocol for a Cochrane Review (Diagnostic test accuracy). The objectives are as follows:

To assess the diagnostic accuracy of rapid diagnostic tests (RDTs) for detecting *P vivax* malaria parasitaemia in people living in malaria-endemic areas who present to ambulatory healthcare facilities with symptoms suggestive of malaria, and to identify which types and brands of commercial tests best detect *P vivax* malaria.

BACKGROUND

Target condition being diagnosed

Malaria is a life-threatening disease caused by *Plasmodium* species (*Plasmodium* spp.), transmitted by the bite of a female *Anopheles* mosquito. Currently, there are five established *Plasmodium* spp. that cause malaria in humans. The two most common are *Plasmodium falciparum* and*Plasmodium vivax*. Vivax malaria is a relapsing form, which is rarely fatal but can cause serious anaemia in children (Abba 2014). There has been an increased focus on *P vivax* as malaria-endemic settings that also have *P falciparum* have made progress in *P falciparum* control. Outside Africa, *P vivax* is the predominant *Plasmodium* spp. in the Americas, South-East Asia, and the World Health Organization (WHO) Eastern Mediterranean regions; causing 64%, greater than 30%, and greater than 40% of all malaria cases, respectively (WHO 2017a). People with malaria caused by *P vivax* can have relapses due to the dormant liver stage hypnozoites. People can carry hypnozoites ranging from a few weeks to more than 12 months before reporting symptoms again (Battle 2014). Primaquine is recommended additionally to standard malaria treatment for *P vivax* and *Plasmodium ovale* to clear these liver stage parasites. Due to this, it is important to have diagnostic tests that are highly sensitive and specifically detect *P vivax* from other *Plasmodium* spp.

Index test(s)

Rapid diagnostic tests (RDTs) (WHO 2003), detect parasite-specific antigens in a drop of fresh blood through lateral flow immunochromatography (WHO 2006). Generally, RDTs do not require a laboratory, any special equipment, or specialized training. They are easy to use and can give results as a simple positive or negative result, at thresholds pre-set by the manufacturers, within

15 to 20 minutes (Talman 2007; WHO 2006). Therefore, RDTs are, in general, suitable for remote areas with limited facilities and lack of laboratory expertise. However, they have a limited shelf life (24 months) and need to be kept dry and away from temperature extremes (greater than 40°C). They may also fail to detect malaria where there are low levels of *Plasmodium* parasites in the blood and false positives are possible due to cross reactions with other disease conditions, presence of certain immunological factors, and gametocytaemia (Kakkilaya 2003).

There is strong evidence that storage conditions of the RDT affect their performance (Moonasar 2007). The parasite density of the blood sample can also affect the performance of the RDT. The WHO malaria RDT product testing programme report investigated the effect of parasite density by testing individual products under laboratory conditions using standardized blood samples at low and high parasite densities (200 and 2000 parasites/μL), and reported the 'panel detection score' (WHO 2012). An existing Cochrane Review on non-falciparum RDTs found that parasite density and storage conditions are often poorly reported in field studies (Abba 2014). Morever, due to the lag period between when the RDT was evaluated by the WHO malaria RDT product testing programme to when the RDT is actually used in the field, manufacturers may have modified the RDT during this period.

Different types of RDT use different types of antibody or combination of antibodies to detect *Plasmodium* antigens. Some antibodies aim to detect a particular species while others are pan-malarial, aiming to detect all types of *Plasmodium*. Currently, all commercial RDTs specific for *P vivax* use *P vivax*-specific lactate dehydrogenase (LDH) antigens (WHO 2017b).

Clinical pathway

People of any age with malaria typically present to medical care with non-specific symptoms of fever, headache, chills, or rigor. The RDTs are most commonly used at the point of presentation with these symptoms, most often in settings where quality microscopy is not available. Parasitological diagnosis is recommended prior to commencing on any treatment (WHO 2015a).

Prior test(s)

It is unlikely that patients will have had previous testing for their current infection prior to presentation to healthcare centres with symptoms of malaria. One key benefit of RDTs is the ease of use at point of care. For the purpose of this review, we will not address the sensitivity or specificity of *P vivax*-specific RDTs for confirming efficacy of treatment as this is not recommended practice.

Role of index test(s)

Malaria is a common cause of fever in endemic regions. Given the non-specific symptoms patients with malaria often present with, a parasitological test is recommended to make a formal diagnosis (WHO 2015b). Often people of any age or gender presenting to a healthcare clinic with a history of fever in a malaria-endemic region will undergo a malaria test as part of a routine initial work-up. As such, the population receiving the index test would be identified solely on the basis of the clinical history and physical examination. RDTs have a role in malaria diagnosis where there is no access to good quality microscopy services and in outbreak investigation or surveys of parasite prevalence. Reliable diagnosis of *P vivax* malaria with RDTs would not only benefit the individual by allowing treatment of the blood stage and latent hypnozoite stage, but also would have benefits at a population level by potentially reducing low-level ongoing transmission due to relapsing disease. Widespread use of accurate RDTs can facilitate greater diagnosis and treatment rates of *P vivax* malaria in areas where there is inadequate access to high-quality microscopy.

True positive results would allow effective treatment of active disease and facilitate prevention of relapse using drugs that target the liver stage hypnozoites such as primaquine or tafenoquine, thus effectively treating individuals and reducing the risk of onward transmission. True negative results facilitate accurate diagnosis by narrowing differential diagnoses of people presenting to care with fever and non-specific symptoms. False positives would potentially lead to over treatment of individuals with primaquine and either chloroquine or artemisinin combination therapies. False negatives would lead to potential relapsing disease and potentially ongoing transmission at the population level.

Alternative test(s)

Microscopic examination of Giemsa-stained thick and thin blood films remains the conventional laboratory method. Microscopic examination has good sensitivity and specificity, and it allows species and stage differentiations and quantification of parasites, all of which are important in assessing disease severity, monitoring response to treatment, and prescribing appropriate therapy. Intensive examination is more likely to reveal parasitaemia so the test is carried out with a fixed number of fields examined. Infections may be missed if slides are not examined carefully (Wongsrichanalai 2007). Very low parasitaemia may be missed even by good quality microscopy; the limit of detection of thick smear microscopy has been estimated at approximately 4 to 20 asexual parasites/μL, although a threshold of 50 to 100 asexual parasites per μL is more realistic under field conditions (Wongsrichanalai 2007). False-positive results are also possible; if blood slides are not prepared carefully, artefacts may be formed, which can be mistaken for *Plasmodium* parasites (Wongsrichanalai 2007).

Polymerase chain reaction (PCR), which is a molecular method based on DNA amplification, is the most accurate method of detecting parasites in the blood. Compared to microscopy, PCR is less prone to observer error and more sensitive at low levels of parasitaemia (Snounou 1993). For PCR, the limit of detection may

be as low as 0.004 asexual parasites/μL (Hänscheid 2002). However, whether this increased ability to detect low level parasitaemia makes it a better diagnostic test is uncertain, as submicroscopic parasitaemia are of unknown clinical significance and the prevalence of asymptomatic submicroscopic infection is high in some areas (May 1999). PCR is currently not widely available due to logistical constraints and the need for specially-trained technicians and a well-equipped laboratory. It is usually used only for research purposes.

Rationale

P vivax is becoming increasingly important, especially in regions targeting malaria elimination. In areas of co-endemicity, vivax malaria is increasing proportionally compared to falciparum malaria. Moreover, treatment for *P vivax* and *Plasmodium ovale* malaria differs from other types of malaria. Therefore, it is important the RDT can correctly identify *P vivax* from other species. Geographically, *P vivax* has a much wider infection range compared to other *Plasmodium* spp and this may increase over time due to climate change (Culleton 2012). Historically, autochthonous transmission of *P vivax* was happening in temperate climates, such as England (Dobson 1994). An existing Cochrane Review assessing RDTs for diagnosing uncomplicated non-falciparum malaria was conducted in 2014 (Abba 2014). A subset of this review included RDTs that diagnosed *P vivax*. This review will only assess the diagnostic accuracy of RDTs that specifically detect *P vivax* with vivax specific lactate dehydrogenase (LDH) antigens.

OBJECTIVES

To assess the diagnostic accuracy of rapid diagnostic tests (RDTs) for detecting *P vivax* malaria parasitaemia in people living in malaria-endemic areas who present to ambulatory healthcare facilities with symptoms suggestive of malaria, and to identify which types and brands of commercial tests best detect *P vivax* malaria.

Secondary objectives

To assess the effect of transmission setting (perennial, seasonal, or epidemic) and type of malaria present in the region on the accuracy of RDTs for detecting *P vivax* malaria parasitaemia. We will also assess the effect of different generations of an RDT on test accuracy. For studies that used microscopy as the reference standard, we will also assess the impact of level of training.

METHODS

Criteria for considering studies for this review

Types of studies

We will include retrospective or prospective cohort or cross-sectional studies that assessed the accuracy of an RDT or compared the accuracy of two or more RDTs in the same study population (i.e. comparative accuracy studies). We will exclude case-control studies because they are known to overestimate test accuracy and we expect to find sufficient studies of a cross-sectional or cohort design. Studies sampling a consecutive series of patients, or a randomly selected series of patients are eligible. Where the report has not explicitly stated that sampling was consecutive, but we judge that consecutive sampling was most probable, we will include the report. We will exclude studies if they did not present sufficient data to allow us to extract or deduce the number of true positives, false positives, false negatives, and true negatives (i.e. 2 x 2 data).

Participants

Studies recruiting people living in *P vivax*-endemic areas attending ambulatory healthcare settings with symptoms of uncomplicated malaria are eligible.

We will exclude studies if participants:

- were not immune to malaria had travelled from non-malarious region to malarious regions, e.g. travellers or displaced populations;
- had been previously treated for their current malaria infection or the test was performed to assess whether treatment was successful, or both;
- had symptoms of severe malaria as defined by the WHO clinical definition (WHO 2014);
- did not have symptoms of malaria as defined by history of fever, headache, or chills/rigor;
- were recruited through active case finding (for example, door to door surveys).

In studies where only a subgroup of participants is eligible for inclusion in the review, we will include the study provided that we can extract relevant data specific to that subgroup. If studies included some patients with severe malaria, and we cannot extract data specific to the subgroup of participants with uncomplicated malaria, we will include the study if 90% or more of the participants had uncomplicated malaria.

Index tests

Studies evaluating any immunochromatography-based RDT specifically designed to detect *P vivax* malaria. We will only include RDTs that meet the WHO malaria RDT performance criteria (WHO 2017b).

Target conditions

Studies that aimed to detect *P vivax* malaria.

Reference standards

Studies are required to diagnose *P vivax* malaria using at least one of the following two reference standards:

- conventional microscopy of thick blood smears and thin blood smears. We will regard the presence of asexual parasites at any density as a positive smear. Once the diagnosis is established - usually by detecting parasites in the thick smear - the laboratory technician can examine the thin smear to determine the malaria species and the parasitaemia, or the percentage of the patient's red blood cells that are infected with malaria parasites. The thin and thick smears are able to provide all three of these vital pieces of information. Ideally, blood smears would be examined independently and in duplicate with more than 100 high-power fields;
- PCR, including quantitative PCR (qPCR), nested PCR (nPCR), and real-time PCR (rPCR). We will also include studies that use loop-mediated isothermal amplification (LAMP). Most PCR-based assays for *P vivax* are only available as laboratory-developed tests, which means they are rarely used clinically outside of research projects where *P vivax* malaria is endemic. They are especially useful for diagnosing asymptomatic people as the assays have high sensitivity, particularly in samples with low density of parasites. Molecular diagnostics theoretically have a lower limit of detection than both RDTs and microscopy depending on the training of microscopists and quality of samples analysed. Significant variation exists between molecular diagnostics developed including type of input material (DNA, RNA, or whole blood), target gene, (number of) species detected, primer/probe composition and concentration, amplification technique (PCR or isothermal), read-out (gel-electrophoresis, fluorescence detection, lateral flow), and whether it is qualitative or quantitative. However, no important differences have been found in the accuracy of these tests (Roth 2016).

For studies that used both reference standards, we will extract 2 x 2 data for each reference standard and stratify the analyses by reference standard.

Search methods for identification of studies

We will attempt to identify all relevant studies regardless of language or publication status (published, unpublished, in press, and in progress).

Electronic searches

We will search the following databases using the search terms and strategy described in Appendix 1: Cochrane Infectious Diseases Group Specialized Register; Central Register of Controlled Trials (CENTRAL), published in the Cochrane Library; MEDLINE (PubMed); Embase (OVID); Science Citation Index Expanded (SCI-EXPANDED) and Conference Proceedings Citation Index-Science (CPCI-S), both in the Web of Science. We will adapt the search terms for MEDLINE (PubMed) listed in Appendix 1 to other electronic databases. We will report all search strategies in full in the final review version.

We will also search the WHO International Clinical Trials Registry Platform (WHO ICTRP; www.who.int/ictrp/en/) and ClinicalTrials.gov (clinicaltrials.gov/ct2/home) for trials in progress, using "vivax malaria", "Plasmodium vivax", and "rapid diagnostic test*" or RDT* as search terms.

Searching other resources

We will check the reference lists of studies identified by the above methods.

Data collection and analysis

Selection of studies

Two review authors (LC and SJ) will independently assess study eligibility by examining the title and abstract of each article identified by the literature search and will exclude obviously irrelevant studies. If either review author considers the abstract to be potentially eligible, we will obtain the full-text article. Two review authors will independently assess each full-text article against the predefined inclusion and exclusion criteria, as discussed in the 'Criteria for considering studies for this review' section, and will resolve any disagreements by discussion. If the review authors cannot reach consensus, a third review author will have the final decision. We will list all articles excluded after full-text assessment and the reasons for exclusion in the 'Characteristics of excluded studies' table. We will illustrate the study selection process using a PRISMA flow diagram.

Data extraction and management

Two review authors (LC and SJ) will independently extract data using a pre designed data extraction form.

We will extract the following data.

- Authors, publication year, and journal.
- Study design.
- Study start date.
- Characteristics study participants (age, gender, co morbidities, and pregnancy).
- Study inclusion/exclusion criteria.
- Study setting.
- Malaria species in study setting.

- Malaria prevalence and endemicity in study setting.
- Reference standard.
- Index test (brand name, target antigen, and batch numbers).
- Additional tests (and their results).
- RDT and reference standard setting.
- Lot testing of RDT used.
- Transport and storage conditions of RDTs.
- Training level of person performing index test.
- Training level of person performing reference standard (and if available the WHO certified training level of the microscopist).
- Number of high power fields observed in microscopy.
- Parasite density of microscopy positive cases.
- Observers or repeats used.
- Number of indeterminate, missing or unavailable test results.
- Number of true positives, false positives, false negatives, and true negatives.
- Type of molecular amplification assay.
- Volume of blood samples.
- Limit of detection for PCR.

We will resolve any discrepancies in data extraction by discussion. If needed, we will consult a third review author. We will contact the authors of primary studies if we cannot resolve any disagreements. In the event that this is unsuccessful, we will report the disagreement in the review.

We will also report key study characteristics across studies in a separate, additional table. These characteristics will include factors which may affect the performance of RDTs (training level of person performing the RDT, storage conditions, whether the RDT used was lot-tested, and parasite density of microscopy-positive cases).

Assessment of methodological quality

We will use the revised tool for the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) to assess the risk of bias and applicability of included studies (Whiting 2011). We have tailored the tool to the context of the review as shown in Appendix 2. Two review authors (LC and SJ) will independently assess methodological quality using the tailored QUADAS-2 tool. We will resolve any disagreements through consensus or by consulting a third review author. We will use both graphics and text to summarize the results.

Statistical analysis and data synthesis

We will stratify all analyses by the type of reference standard used. We will perform preliminary analyses by plotting estimates of sensitivity and specificity from the included studies on forest plots and in receiver operating characteristic (ROC) space using the software, Review Manager 5 (RevMan 5) (RevMan 2014).

If there are sufficient data, we will perform meta-analyses using bivariate models to estimate and compare summary sensitivities and specificities (summary points) (Chu 2006; Macaskill 2010; Takwoingi 2015b).

To provide a coherent set of comparative analyses, we will group studies according to commercial brand within each RDT type so that in the primary test comparison we make comparisons between RDT types and then between the brands of each RDT type separately in secondary analyses. We will perform the test comparisons using two analytic strategies: indirect and direct comparisons. Indirect comparisons will be performed by using all available studies because comparative studies are known to be generally scarce (Takwoingi 2013). However, indirect comparisons are prone to bias due to confounding (Takwoingi 2013). Therefore, we will also perform direct comparisons if comparative studies that make head-to-head comparisons of brands or types in the same population are available. We will compare test accuracy by including covariate terms in bivariate models to estimate differences in sensitivity and specificity. We will assess the statistical significance of these differences by using likelihood ratio tests to compare models with and without the covariate terms.

If analyses using bivariate models fail to converge due to sparse data or few studies, we will simplify the models to univariate random effects logistic regression models to pool sensitivity and specificity separately (Takwoingi 2015a). We will perform meta-analyses using the meqrlogit command in Stata (STATA 2015).

Investigations of heterogeneity

Following preliminary investigations on forest and SROC plots, if there are sufficient data, we plan to formally investigate heterogeneity by adding each potential source of heterogeneity as a covariate to a bivariate model (i.e. meta-regression). We plan to assess the effect of transmission setting (perennial malaria, seasonal, or epidemic) and type of malaria in region (vivax only, vivax and falciparum, mixed non-falciparum, or all types). To investigate the effect of different generations of an RDT as a result of modifications made by a manufacturer, if explicit information is unavailable, we will use study start date as a proxy and include it as a covariate in a bivariate model. For studies that used microscopy as the reference standard, we will assess the impact of level of training as defined in conduct of the reference standard as according to QUADAS-2 (Appendix 2).

Sensitivity analyses

If there are sufficient data, we will perform sensitivity analyses to examine the influence of risk of bias and study design (i.e. retrospective and prospective study designs on test accuracy). We will examine the effect of including only studies that used a reference standard that was likely to correctly classify the target condition by excluding studies at high or unclear risk of bias from the main

analyses. We will also assess the impact of including only studies where participants received the same reference standard.

Assessment of the certainty of the evidence

We will assess the certainty of the evidence (i.e. quality of evidence or confidence in effect estimates) using the GRADE approach and GRADEpro Guideline Development Tool software (GRADE 2013; GRADEpro GDT 2015). In the context of a systematic review, the ratings of the certainty of the evidence reflect the extent of our confidence that the estimates of test accuracy are correct. As recommended, we will rate the certainty of the evidence as either high (not downgraded), moderate (downgraded by one level), low (downgraded by two levels), or very low (downgraded by more than two levels) for four domains: risk of bias, indirectness, inconsistency, and imprecision. For sensitivity and specificity, the certainty of the evidence will initially start as high when there are high-quality cross-sectional or cohort studies that enrolled participants with diagnostic uncertainty. If we find a reason for downgrading the certainty of the evidence, we will classify the reason as either serious (downgraded by one level) or very serious (downgraded by two levels).

We will describe the implications of sensitivity and specificity in terms of individual and population level for each of true positives. Two review authors (LC and SJ) will discuss judgments and reach a consensus. We will apply GRADE in the following way.

- Risk of bias: we will use QUADAS-2 to assess risk of bias.
- Indirectness: we will consider indirectness from the perspective of test accuracy. We will use QUADAS-2 to assess applicability concerns and look for important differences between the populations studied (for example, in the transmission intensity as defined by the WHO World Malaria Report or WHO malaria country profiles for the corresponding year), the setting, and the review question.
- Inconsistency: GRADE recommends downgrading for unexplained inconsistency in sensitivity and specificity estimates. We will carry out pre-specified analyses to investigate potential sources of heterogeneity and will downgrade when we cannot explain inconsistency in the accuracy estimates.
- Imprecision: we will consider the width of the confidence intervals (CIs), and ask ourselves, "would we make a different decision if the lower or upper limit of the 95% confidence interval (CI) represented the truth?". In addition, we will calculate absolute numbers of true positives, false negatives, false positives, and true negatives, as well as ranges for these values based on the CIs of the pooled estimates of sensitivity and specificity for various prevalences of vivax malaria; we will also make judgements on imprecision using these calculations.

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* *Indicates the major publication for the study*

APPENDICES

Appendix 1. Detailed search strategy

Search set	MEDLINE (PubMed)
1	Malaria, vivax [MeSH]
2	Plasmodium vivax [MeSH]
3	"Plasmodium vivax" or "P vivax" or "vivax malaria" or "non-falciparum Malaria" Field: Title/Abstract
4	1 or 2 or 3
5	Exp Reagent kits, diagnostics [MeSH]
6	"Diagnostic Tests, Routine"[Mesh]
7	rapid diagnostic test* Field: Title/Abstract
8	RDT* Field: Title/Abstract
9	Dipstick* Field: Title/Abstract
10	"Rapid diagnostic device*" Field: Title/Abstract
11	MRDD Field: Title/Abstract

(*Continued*)

12	OptiMal Field: Title/Abstract
13	"Binax NOW" or "NOW-ICT-Malaria" or "NOW-Malaria-ICT" Field: Title/Abstract
14	ParaSight or Parascreen or ParaHIT Field: Title/Abstract
15	"SD Bioline" or Carestart or Falcivax or Malascan Field: Title/Abstract
16	Immunochromatograph* or Immuno-chromatograph* Field: Title/Abstract
17	"Antigen detection" Field: Title/Abstract
18	"Rapid malaria antigen test*" Field: Title/Abstract
19	"Combo card test*" Field: Title/Abstract
20	Immunoassay [MeSH]
21	Chromatography [MeSH]
22	Enzyme-linked immunosorbent assay [MeSH]
23	"Rapid test*" Field: Title/Abstract
24	"Card test*" Field: Title/Abstract
25	Rapid AND (detection* or device* or test* or kit*) Field: Title/Abstract
26	5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25
27	4 and 26

Appendix 2. QUADAS-2 tool tailored to the context of the review

Domain	**Patient selection**	**Index test**	**Reference standard**	**Flow and timing**
Description	**Methods of patient selection**	**How index test was conducted and reported**	**How reference standard was conducted and reported**	**Describe patients that did not receive and time interval between index test or reference standard**
Signalling questions (yes, no, or unclear)	Consecutive or random sample of patients? • 'Yes' if the study	Index test results interpreted without knowledge of the results of ref-	**PCR** PCR likely to correctly classify the target condi-	Was there an appropriate interval between index

(*Continued*)

	reported consecutive enrolment or random sampling of patients presenting with uncomplicated malaria symptoms. • 'No' if patients were purposefully selected, for example based on previous test results (such as using Rapid diagnostic tests (RDTs) only on those who tested positive for *P vivax* by microscopy/ PCR). • 'Unclear' if the study did not explicitly state consecutive enrolment or random sampling, and it was unclear how patients were sampled.	erence standard? • 'Yes' if RDT was performed fully blinded to reference standard result. • 'No' if reference standard result was known prior to interpretation of RDT result. • 'Unclear' if blinding was no explicitly stated.	tion? We will answer this question as 'yes' for all studies because PCR is an objective test with binary outcomes. Thus, there is no room for subjective interpretation of test results or poor performance of the test leading to false negatives or false positives 'Yes' if reference standard was PCR. **Microscopy** Microscopy likely to correctly classify the target condition? 'Yes' if microscopy was performed for one sample by two independent trained microscopist examining 100 high-power fields 'No' if microscopy was performed: • by insufficiently trained individuals; • by one individual only; • with inadequate equipment; • by viewing less than 100 microscopic fields before declaring negative. 'Unclear' if insufficient information was provided.	test and reference standard? • 'Yes' if samples for RDT and microscopy or PCR were taken at the same time. We felt this was important given the transient parasitaemia associated with malaria. • 'No' if the samples for RDT and microscopy or PCR were taken at different times. • 'Unclear' if insufficient or no information on the time interval.
	Was a case-control design avoided? This will always be 'yes' because case control studies will be excluded from this review	Pre-specified threshold used? As the threshold is pre-specified by the manufacturer in all RDTs, we will answer this question 'yes' for all studies	Reference standard results interpreted without knowledge of the results of index test? We will answer this question 'yes' for all studies using only PCR as the reference standard be-	Did all patients receive a reference standard? • 'Yes' if all participants received a microscopy or PCR. • 'No' if one or more participants did not receive microscopy or

(*Continued*)

			cause PCR is an objective test with binary outcomes. Thus, there is no room for subjective interpretation of test results • 'Yes' if results of microscopy were interpreted without knowledge of RDT results • 'No' if results of microscopy were interpreted with knowledge of RDT results • 'Unclear' if there is insufficient information on whether or not microscopy results were interpreted with knowledge of RDT results	PCR. Or if the reference standard was applied depending on index test results • 'Unclear' if there is insufficient information to determine whether or not all patients received microscopy/PCR.
	Did the study avoid inappropriate exclusions? • 'Yes' if no patients were excluded after inclusion in the study or if exclusions are adequately described. • 'No' if specific populations were excluded (for example, pregnant patients, children or immunocompromised patients), • 'Unclear' if unreported or insufficient information given to make a decision.			Did all patients receive the same reference standard? • We will answer this question 'yes' if all participants in the study or a subset of participants in the study received the acceptable reference standard (microscopy, PCR, or both), which we specified as a criterion for inclusion in the review. • 'No' if participants did not receive the same reference standard. • 'Unclear' if there is insufficient information to determine whether or not all patients received the same reference standard.
				Were all patients included in the analysis? • 'Yes' if the number of participants in the two-by-two table

(*Continued*)

				matches the number of participants recruited into the study or if sufficient explanation was provided for any discrepancy. • 'No' if some participants recruited into the study were unaccounted for. • 'Unclear' if unreported or insufficient information given to make a decision.
Risk of bias (high, low, or unclear)	Could the selection of patients have introduced bias?	Could the conduct or interpretation of the index test have introduced bias?	Could the reference standard, its conduct, or its interpretation has introduced bias?	Could the patient flow have introduced bias?
Applicability concerns (high, low, or unclear)	Are there concerns that the included patients do not match the review question? • 'High' if the participants included in the study lived in an area with high prevalence of *P vivax* malaria. • 'Low' if participants included in the study lived in an area with low or very low prevalence of *P vivax* malaria. • 'Unclear' if insufficient information to make a decision.	Are there concerns that the index test, its conduct, or interpretation differs from the review question? • 'High' if the study describes inappropriate storage conditions for the index test, or if the index test has not been lot tested • 'Low' if the study describes suitable storage conditions for the index test that meet manufacturer's requirements and if the study has reported the index test has been lot tested • 'Unclear' if insufficient information to make a decision	Are there concerns that the target condition as defined by the reference standard does not match the review question? We will answer this question 'low' for all studies because *P vivax* diagnosed by light microscopy or PCR does match the review question	Not applicable

CONTRIBUTIONS OF AUTHORS

LC and SJ drafted the protocol. YT and JC provided methodological input, content expertise, and assessed the protocol draft. All review author read and approved the final protocol draft.

DECLARATIONS OF INTEREST

LC has no known conflicts of interest.

SJ has no known conflicts of interest.

JC has partial salary support from the Bill and Melinda Gates Foundation through the World Health Organization (WHO), and has no conflicts of interest to declare.

YT has no known conflicts of interest.

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