World Health Organization

Preferred product characteristics: tests for risk of *P. vivax* relapse

Draft for public consultation

Table of Contents

Overview 2

Terminology 2

Background 3

Natural history of *P. vivax* infection 3

Public health response and key challenges 4

Available diagnostic tools for detecting clinical vivax infection 5

Current landscape for tests for risk of *P. vivax* relapse or hypnozoite carriage 5

WHO strategic goals for tests for *P. vivax* relapse 6

PPC 1. Point of care test for risk of *P. vivax* relapse 8

PPC 2. Laboratory based test for risk of *P. vivax* relapse 15

Annex 1. Definition of health system infrastructure levels 22

Overview

The *Global technical strategy for malaria 2016–2030* (GTS) aims to harness and expand research to accelerate progress towards the elimination of malaria and to counteract the emerging threat of drug and insecticide resistance.[1] It encourages innovation and the development of new tools, technologies and strategies (collectively referred to as 'interventions') to maintain progress in malaria control and to further advance towards elimination. To accelerate implementation of the GTS, the World Health Organization's (WHO) Global Malaria Programme (GMP) conducted a review of its guidelines and guidance development processes, to ensure transparency, consistency, efficiency, and predictability. One of the outcomes of the review was the adoption of "preferred product characteristics" (PPCs) to incentivize and guide the development of urgently needed health products. The use of PPCs is aligned with an organization-wide effort to improve WHO's communication on identified public health needs and to encourage and facilitate innovation to meet those needs.

WHO PPCs aim to:

- communicate unmet public health needs;
- stimulate the development of relevant new products to meet those needs; and
- facilitate the timely, effective assessment of new products, and the formulation of WHO recommendations and prequalification listings.

Terminology

PPCs are designed to communicate unmet public health needs identified by WHO, stimulate innovation and investment in the identified areas, and communicate the desired performance and operational characteristics of health products to address those needs. The target audience consists of product developers including researchers, regulatory agencies, procurement agencies, and funders of research and development. PPCs are usually developed before a mature pipeline of products is available and should reflect the ideal characteristics of interventions required to rapidly and effectively achieve public health impact.

Target Product Profiles (TPPs) in the context of public health are used to set research and development targets for manufacturers and researchers to guide the development of specific products. TPPs provide more detailed information than PPCs and include both minimally acceptable and preferred performance characteristics. The minimum performance characteristics should be considered a "go/no-go" decision point in the product development process.

Background

Relapsing malaria is a mosquito-borne parasitic infection caused by two species of human parasites, *Plasmodium vivax* (*P. vivax*) and two subspecies of *Plasmodium ovale* (*P. ovale*). *P. vivax* malaria is endemic in all WHO regions except EURO, with an estimated 4.5-5.5 million clinical cases in 2021.[2] *P. vivax* is an integral target of malaria elimination efforts, but also remains a substantial cause of morbidity and contributor to childhood mortality in endemic regions. *P. ovale* is a sporadic infection in Africa and the Asia-Pacific with a very limited clinical burden.[3]

The WHO Global Malaria Programme and other organisations and stakeholders have called for the global reduction of malaria burden towards eventual malaria elimination.[1,4] As the major relapsing malaria species, *P. vivax* malaria presents a major challenge to achieving these targets. About 1/3 of the global population is still at risk of contracting *P. vivax* malaria. The formation of dormant hypnozoites by *P. vivax* means that tailored and sustained interventions are required to control the burden of this species and their impact is slower than against *P. falciparum*. Therefore, as transmission declines in co-endemic areas, *P. vivax* becomes the main source of clinical malaria and foci of stable (high) *P. vivax* transmission can persist even when *P. falciparum* is nearing elimination.

Around 85% of *P. vivax* clinical/blood-stage infections are due to reactivation of latent parasites, rather than newly transmitted infections.[5,6] While less often associated with acute illness, continued relapses are a cause of chronic anaemia[7] and have been shown to be associated with an excess in morbidity and mortality.[8] In addition to diagnostic tools that can detect the acute, erythrocytic phase of *P. vivax*, new tools are needed to detect dormant infections before they reactivate and contribute to morbidity and onward transmission.

To help guide research and development efforts and assist donors, technical agencies and ministries of health to select products that best respond to public health needs, the WHO Global Malaria Programme, with input from a Preferred Product Characteristics Development Group made up of clinicians, public health experts and laboratory scientists, has developed two prioritized preferred product characteristics (PPCs) for tests to detect risk of *P. vivax* relapse. These tools are expected to improve screening, use of radical cure and case management for high-risk populations, as well as support population-level risk stratification for targeting of interventions and monitoring and evaluation of ongoing elimination programmes.

The PPCs submitted for public consultation describe 2 types of tests for risk of *P. vivax* relapse:

- The first is a point-of-care test to identify individuals at risk of *P. vivax* relapse to guide radical cure and case management (PPC 1). This is based on detection of analytes indicative of hypnozoite carriage and/or current sequestered infection and/or recent (blood borne) infection with *P. vivax*.
- The second is a laboratory-based test to identify communities or individuals at risk of *P. vivax* relapse (PPC 2). Unlike the POC test (PPC 1), this test will be used to screen large numbers of individuals simultaneously as part of surveillance and/or monitoring activities related to *P. vivax* control and elimination.

Natural history of *P. vivax* infection

The distinguishing feature of *P. vivax* malaria is its establishment of a latent infection in the liver within a couple of days of infection. After sporozoite invasion of the hepatocyte, it undergoes rapid transformation into either a rapidly developing, metabolically highly active liver merozoite or into a developmentally arrested (called "dormant" as follows) hypnozoite. Microscopy and antigen-detecting rapid diagnostic tests (RDT) detect only parasites or their antigens, respectively, which are released into the blood stream (erythrocytic phase) and therefore cannot detect the presence of

hypnozoites. The dormant hypnozoites are refractory to the radical cure drugs which kill active liver-stage infection (atovaquone, proguanil, pyrimethamine) and blood-stage infections. The triggers for hypnozoite re-activation and the re-establishment of blood parasitaemia remain unclear. However, *P. falciparum* infection is one condition that appears to be involved. Both species are transmitted by the same mosquitoes, and individuals who have been exposed to *P. falciparum*-carrying mosquitoes in the last 3-4 weeks are also at high(er) risk of having encountered *P. vivax*-infected mosquitoes. This co-exposure means that *P. falciparum* cases have a much higher risk of carrying hypnozoites compared to the general population.[9,10]

While relapse patterns are vivax strain specific and hence vary by geography, epidemiological studies have shown most clinical relapses occur within two years, and rarely after four years. In the tropics, relapses tend to be at shorter intervals, with tropical and sub-tropical strains experiencing a first relapse within a few weeks to 6 months after the primary blood-stage infections. Subsequent relapses tend to be more frequent. The proportion of *P. vivax* infections leading to relapses is also highly variable, determined in part by sporozoite inoculum and immunity.[2] Rarely, all the sporozoites invading a liver become hypnozoites and none form liver schizonts that directly progress to a blood infection and febrile illness (called *P. vivax hibernans*, now limited to the Korean peninsula).

The relapsing pattern of *P. vivax* and gametocyte production are key transmission determinants for this parasite. Unlike *P. falciparum,* which can sustain a chronic blood-stage infection by repeatedly changing the surface coat of blood merozoites to avoid the host immune response, a blood-stage infection of vivax has no comparable immunological escape mechanism and is therefore vulnerable to immune suppression and clearance. Regular relapses are thus required for the maintenance of chronic *P. vivax* infections.

Public health response and key challenges

A 2015 WHO monograph, *Confronting* P. vivax *malaria*,[11] outlined key gaps in our toolbox against vivax malaria, many of which persist despite increased efforts to develop *P. vivax*-specific tools to address them. These gaps include:

1. The lack of vector control tools against outdoor biting/resting mosquitoes, which often transmit vivax.
2. Compared to *P. falciparum*, the number of parasites circulating in the blood of a person infected with *P. vivax* malaria is typically lower. Therefore, a greater proportion of *P. vivax* infections may be missed by current case management diagnostic tools (antigen detecting RDTs and microscopy) even if a patient presents with symptoms.
3. It has been recognised that the majority of *P. vivax* transmission is due to asymptomatic infections (low density blood stage or sequestered infection in the blood or bone marrow, for example). This highlights the need to seriously consider new strategies and new tools for identifying blood stage infections and safely treating asymptomatic / afebrile carriers if *P. vivax* transmission is to be reduced expeditiously in line with national and regional targets.
4. Detection of latent *P. vivax* infection. As described above, the dormant liver stage infections of vivax cannot be directly detected, and there is a large reservoir of people who are infected but unaware of their condition.
5. Suboptimal *P. vivax* radical cure treatment regimens. Treatment today requires a 7- or 14-day course of primaquine to treat a dormant infection, although 1-day tafenoquine is now registered in several countries and is in pilot implementation. In the absence of effective radical cure treatment, people are at risk of multiple clinical relapses and its associated morbidity and mortality in young children.

6. Affordable, accurate and near-patient tests for G6PD deficiency to stratify which persons are at risk for clinically significant 8-aminoquinoline-induced haemolysis. WHO recently developed target product profiles for tests of G6PD activity to support safe and effective anti-relapse therapy for *P. vivax*[2] and at least one G6PD diagnostic is being evaluated in operational research studies to guide use of tafenoquine or high-dose primaquine.

Since 2015, it has been recognised that *P. vivax* causes cryptic, asexual infections in a person's spleen and bone marrow, posing additional challenges. Specifically, *P. vivax* seems to be taking advantage of the splenic reservoir of immature reticulocytes and the majority of its lifecycle can take place in the spleen.[12,13] Bone marrow infections are associated with dyserythropoiesis and inefficient erythropoiesis.[14] Although many of these infections are associated with (very) low density blood-borne parasitaemia, some are undetectable even by highly sensitive PCR.[12,13] Tests that could identify this hidden reservoir would also be a proxy for probable hypnozoite carriage and risk of *P. vivax* relapse.

In essence, implementation of health-setting appropriate and sufficiently sensitive tests for both blood-stage and latent *P. vivax,* combined with G6PD activity tests, are a prerequisite towards the most effective use of novel antimalarial drugs against *P. vivax* malaria (like tafenoquine) beyond treatment of acute symptomatic/febrile patients.

Available diagnostic tools for detecting clinical vivax infection

WHO currently requires that RDTs targeting detection of *P. vivax* achieve a panel detection score[i] of ≥75% at 200p/µL based on the independent laboratory evaluation conducted as a component of the WHO prequalification process.[15,16] This requirement ensures that RDTs will detect the majority of *P. vivax* clinical (symptomatic) infections but the proportion that are missed depends very much on the local epidemiology and, compared to *P. falciparum,* a greater proportion of cases are missed using this threshold.[17] The pyrogenic threshold for vivax may be below 200 parasites/µl,[18] where current RDTs may show reduced sensitivity. The ability of commercial RDTs to meet this target of 200p/µL has increased significantly over the past 15 years, but more data is still needed, particularly for RDTs targeting *P. vivax* specific LDH.[16,19] Due to these real or possibly perceived limitations, some countries, particularly in South and Central America, continue to rely on microscopy. Improving sensitivity of RDTs for *P. vivax* is an ongoing R&D priority, with new assays targeting the lower limits of the pyrogenic threshold. Molecular assays are currently in development for use in point-of-care settings, but for now remain laboratory platforms primarily used for high-throughput surveillance or research applications.

More sensitive tests for *P. vivax* will improve diagnosis of blood-stage infection and may help detect some cryptic *P. vivax* infections in spleen and bone marrow, but there remains a lack of tests detecting latent infection/hypnozoites of *P. vivax* that result in relapse.

Current landscape for tests for risk of *P. vivax* relapse or hypnozoite carriage

As elucidated above, there is an individual and public health need for a test to identify those persons with viable hypnozoites, or a high likelihood of viable hypnozoites, who are therefore at risk of *P. vivax* malaria relapse and represent a potential source of onward transmission. There are no commercial assays that detect hypnozoites or cryptic/sequestered infections; however, there are antibody detecting assays (lateral-flow RDT, indirect immunofluorescence (IFA) or enzyme-linked immunosorbent assay (ELISA)) often used in blood donor screening, that can detect past exposure. These commercial assays are not specific for *P. vivax* and may detect historic infections, whereas the primary interest is in detecting and treating infections acquired over the past 6-9 months, when tropical and subtropical *P. vivax* strains are expected to relapse. R&D efforts are underway such as

[i] Developed to reflect both product sensitivity and reproducibility. It requires four tests, two from each of two manufacturing lots, against the same sample (at 200 parasite/µL) to be positive to register as "detecting" the sample, and quantifies the percentage of samples the product detected.

direct detection of biomarkers of viable hypnozoites, but none have not been successful so far. Methods towards *P. vivax* hypnozoite-derived exosomes are in early biomarker discovery.[20] Indirect measures such as detection of short-lived (6-9 months) immunological responses to *P. vivax* blood-stage antigens, however, have achieved proof-of-principle to detect recent *P. vivax* infection and a high likelihood of relapse.[21,22,23]

WHO strategic goals for tests for *P. vivax* relapse

We envision two major public health value propositions for *P. vivax* relapse assays:

Use case 1: diagnosing relapse risk to guide radical cure

- 1a: Screening and radical cure for high-risk communities in areas targeted for elimination
- 1b: Improving acute case management through providing targeted radical cure after *P. falciparum* to prevent potential *P. vivax* relapses triggered by the *P. falciparum* infection or co-infection. By detecting patients who are likely to also carry hypnozoites at the time that the acute *P. falciparum* infection is diagnosed, targeted treatment can be offered to prevent these relapses.
- 1c: Prevention of re-introduction by screening travelers/ migrants for hypnozoite carriage/risk of relapse (serological/hypnozoite-guided terminal prophylaxis): Identifying individuals at risk of relapse and subsequent reintroduction of *P. vivax* into elimination areas could be averted through screening and treatment.

Use case 1 is of relevance to National Malaria Control Programs (NMCPs) engaged in *P. vivax* elimination. Detection and treatment of those persons with hypnozoites, sequestered infection or a recent *P. vivax* infection as a proxy for the extant *P. vivax* infectious reservoir should reduce time to elimination and will likely prove cost-effective compared to a longer elimination tail.[6] A test detecting relapse risk (through detection of either recent *P. vivax* exposure, sequestered infection or direct evidence of hypnozoite carriage) would prove valuable in proactive or reactive testing particularly in remote settings to detect those subjects likely to harbour hypnozoites. Anyone positive could be treated with safe and effective radical cure, thereby protecting both the individual from the impact of future relapses and reducing the overall community-level parasite reservoir.

In regions with significant co-endemicity of *P. vivax* and *P. falciparum*, many studies have shown that patients with *P. falciparum* have a high risk of subsequent *P. vivax* clinical episodes.[10] This suggests that empiric radical cure may be beneficial to individuals with any type of malaria in co-endemic settings. However, due to concerns with 8-aminoquinoline safety, many vivax endemic countries are reluctant to consider presumptive/empiric vivax radical cure. Confirmation of recent *P. vivax* infection would therefore be a prerequisite to determine appropriateness of radical cure in non-vivax clinical cases. This is particularly important in regions with low *P. vivax* endemicity, where persons with confirmed *P. falciparum* infection are a recognised high-risk population warranting additional testing with a risk of *P. vivax* relapse test as a more informed approach over empiric *P. vivax* radical cure without knowledge of the patient's *P. vivax* status.

A relapse-risk point-of-care test would be preferred over a benchtop test for use case 1 scenarios, as it allows diagnosis and treatment in a single contact. A higher throughput benchtop version would greatly increase the logistical complexity of any screen and treat intervention and suffer from loss to follow-up. The test procedures will also need to be such that minimal operator input is needed, while the test performance (sensitivity / specificity) has to be adequate to justify a screen-and-treat intervention. Test throughput can be modest but should be scalable, if needed. In general, a lateral flow test/RDT format as used for malaria antigen testing can be seen as an example (but not limiting) of an adequate point-of-care test satisfying the above listed criteria.

Use case 2: population-based screening of recent *P. vivax* infections/ hypnozoite carriage for programmatic applications

- 2a: Risk stratification and subsequent targeting of interventions
- 2b: Monitoring and Evaluation (M&E) of ongoing elimination programs

These use cases would be relevant for NMCPs both in near- and post-elimination settings. Such an assay could assist in guiding, monitoring and evaluating the progress of elimination activities by stratifying areas according to likelihood of continued local transmission (pre-elimination) and/or confirming the (continued) absence of local transmission (post-local elimination). Use case 2 needs a higher-throughput assay making more centralised use economical. The test would also be expected to generally exhibit advanced clinical performance, and for use in M&E scenarios, the test should allow for electronic data storage and transmission. On the other side, using a high-throughput lab-based assay for screen-and-treat may be feasible in some settings but would make the intervention much more logistically difficult to implement than with a point-of-care test.

The preferred product characteristics for risk of *P. vivax* relapse tests well suited to these use case scenarios are described in this PPC document.

Table 1: Overview of use scenarios for tests for risk of *P. vivax* relapse

Use scenario	**Screen and radical cure**	**Improve acute case management in *Pf* and *Pv* endemic areas**	**Travelers/migrants entering countries preventing re-introduction**	**Risk stratification (+/- document absence of transmission)**	**Monitoring and evaluation**
Problem addressed	Identifies individuals at risk of *P. vivax* relapse	Identifies individuals at risk of *P. vivax* relapse	Contribute to prevention of re-introduction	Identifies where transmission is happening and at what level	Determines the impact of an intervention either new or established
Target population	High risk communities targeted for elimination	Pf confirmed cases likely to carry hypnozoites	Travelers/migrants	Risk populations	Communities in areas with ongoing elimination programs
Action taken based on result	G6PD test, followed by appropriate 8-aminoquinoline regimen	ACT + G6PD test, followed by appropriate 8-aminoquinoline regimen	G6PD test, followed by appropriate 8-aminoquinoline regimen	Mapping of risk exposure to guide intervention i.e., mass drug administration	Reporting on impact, informing policy
Operational use of implementation	Village	Village	Ports of entry	National, subnational, district	Subnational, district
Transmission level	Low to approaching zero	Low transmission	Zero transmission	Medium to very low	Low to approaching zero
Requires point of care testing	Yes	Yes	Yes	No	No

Table adapted from Ding XC, Ade MP, Baird JK, Cheng Q, Cunningham J, Dhorda M, et al. (2017) Defining the next generation of Plasmodium vivax diagnostic tests for control and elimination: Target product profiles. PLoS Negl Trop Dis 11(4): e0005516. https://doi.org/10.1371/journal. pntd.0005516

PPC 1. Point of care test for risk of *P. vivax* relapse

	Characteristics	Background	Additional notes
1. General requirements			
1.1. Intended use	To identify individuals at risk of *Plasmodium vivax* (*P. vivax*) relapse based on detection of analyte(s) indicative of *P. vivax* hypnozoite carriage and/or current sequestered infection and/or recent (blood borne) infection with *P. vivax*.	Emerging data shows *P. vivax* infection in sequestered sites of erythropoiesis (bone marrow, spleen and liver) in infectious individuals who may carry a high relapse risk.[12,13] "Recent infection" could refer to a previous 9-month window, based on available data from human infection studies and epidemiological studies.[9] The test is designed for being conducted at the point of contact with a patient (in a facility or in the community) or a community member (e.g. during a campaign-style intervention). The rapid turnaround time to result allows for a "screen and treat" approach.	
1.2. Targeted population	All individuals living in *P. vivax* endemic settings where the transmission level ranges from low to elimination, and who are suspected of having latent or sequestered *P. vivax* infection.	A "screen and treat" approach will be applied for those identified to be at risk of *P. vivax* relapse: if this test is positive then it may inform radical cure treatment in line with WHO or national treatment guidelines.	
1.3. Lowest infrastructure level	Test design and procedure allow for use in "low-infrastructure" conditions (Level 0); no cold chain, minimal or no additional laboratory equipment and technical accessories are required.	See Annex 1: Definition of health system infrastructure levels.	

1.4. Lowest level user	The test can reliably be performed by health personnel, community health workers or alike.	The test would ideally be usable by the level of health workers currently performing malaria testing.	
1.5. Test training requirements	≤1 day with Instructions for Use (IFU) and quick reference guide.	Training requirements will vary according to the test format and experience of the users. Consider option for smart phone application(s) to ensure ongoing compliance and up-to-date training.	
2. Design			
2.1. Portability	All supplies required for sample collection and testing procedures packaged with the tests (e.g. lancet, alcohol swabs etc.) If the test setup requires an instrument, it is easily portable (handheld or on desktop, weight <3kg) and no special transport conditions are required (temperature, humidity, vibration, etc.).		
2.2. Instrument / power requirements, if applicable	Battery or solar power operated. No additional equipment required beyond the diagnostic instrument (e.g. micropipettes, vortex, etc.).	May use rechargeable batteries; may also have capacity to be powered by mains supply in addition to portable options.	
2.3. Sample type / collection	Fresh capillary blood from finger sticks and venous blood, or other non-invasive/ minimally-invasive biological samples collected according to routine procedures.	Non-invasive or minimally-invasive samples might include saliva, urine or volatile organic compounds etc.	
2.4. Test procedure	Sample preparation steps should be limited in number and straightforward	Example of sample prep could be one preset dilution or a reagent mixing step. The test	

	to perform, ideally the procedure would be a single step. Transfer of sample specimen to the testing device, either directly or by use of a device provided with the kit (e.g., inverted cup, transfer loop, autofill pipette etc.).	procedure should tolerate a short time lag between sample collection and testing.	
2.5. Sample volume	A volume of sample that can be obtained in a non-invasive/minimally-invasive way in all age groups.	For example, in the case of finger-prick sample, acceptable volume would be <50µL (equivalent to approx. 1 drop)	
2.6. Target analyte	Marker or combination of markers identifying individuals at risk of *P. vivax* relapse.	Risk of relapse may be determined through detection of analytes that indicate (directly and/or indirectly) hypnozoite carriage: 1. Direct evidence: Hypnozoite metabolic markers, or other direct indicator 2. Proxy indicator: Immunological biomarker profiles against *P. vivax* to reflect acute or recently cured blood stage infection (e.g. in the last nine months, comparable to observed relapse patterns). A multiplexed approach interrogating several markers may enhance clinical diagnostic performance. 3. Proxy indicator: Detection of acute sequestered *P. vivax* infection (e.g. spleen, bone marrow) not detectable by current antigen based RDTs or by microscopy Proxy indicator 3 may be insufficiently sensitive and may need to be combined with evidence from 1 or 2.	
2.7. Detection	Unambiguous test interpretation.	For example, via high contrast test line detected via naked eye or, if required, by an instrument; indoor	

		and outdoor reading of a signal that provides a "yes/no" qualitative or a quantitative result	
2.8. Quality Control	Built-in process control indicator (test control).	Tests will need to be manufactured under stringent conditions (i.e. ISO 13485:2016), thus the test is not dependent on positive analyte controls being used for quality control by the user.	
2.9. Supplies needed	Reagents and supplies included in the kit are associated with minimal import restrictions.	Preferably animal-free, no BSA, and no triton X-100, for example.	
2.10. Safety	Safe to both patient and user. Does not expose them to any unnecessary risks. Normal use does not create any additional hazards to the operator when observing Universal Blood Safety precautions	For example, in the case of finger-stick sampling, an auto-retracting sterile lancet should be provided.	
3. Performance			
3.1. Species differentiation	Ideally detects *P. vivax* only.	Interference with other *Plasmodium* species may be acceptable given the low *P. vivax*-endemicity of the intended-use settings and given that mixed-species infections may be present.	

3.2. Diagnostic / clinical sensitivity	> 80% sensitivity to detect a future relapse event	Diagnosis in this use-case informs treatment decisions, so test performance metrics are around individual-level relapse risk detection and aim to reduce over-treatment (relative to the only currently available option of MDA) whilst	Results from recent field studies and modelled impact scenarios assess trade-off between over-treatment with different mass-test and treat strategies and MDA in terms of

		maintaining a public health and individual clinical benefit.[ii] The reference is the occurrence of a *P. vivax* relapse in the months following testing. Successfully detecting all blood-stage relapses is challenging and a high-sensitivity assay (e.g. PCR) should be used to repeatedly test individuals with high regularity. Well-characterized biobanked samples with accompanying metadata (including clinical histories and infection status by PCR) could be used to assess diagnostic performance.	diagnostic sensitivity and specificity.[22,23] WHO encourages development of open biobanks that would facilitate R&D for tests designed to meet PPC targets.
3.3. Diagnostic / clinical specificity	> 90% specificity to detect a future relapse event	Same comments as above	Same comments as above
3.4. Time to results	< 30 minutes	< 30 minutes if multiple samples can be run in parallel; < 60 mins may be acceptable	
3.5. Throughput	≥ 4 tests / hour / operator		
3.6. Target shelf life / storage conditions	≥ 18 months at ≥ 35°C and 90% relative humidity; and able to support short periods of thermal stress	Requirements relate to test kits (i.e., consumables) that are used in the field.	
3.7. Ease of use	Maximum of one timed step; three or less user steps, instructions should include diagram of method and results interpretation; must be usable in an unprotected external environment.		
3.8. Ease of results interpretation	Interpreted by unaided eye or automated readout that is visible in full sunlight		

[ii] In low transmission settings, the positive predictive value of these tests may still to be low. Programmes may need to assess the risk-benefit on a case-by-case basis to balance public health value and clinical benefit to the individual.

3.9. Operating temperature	15°C to 40°C	Operating temperature conditions should be adapted to the use-case and to local conditions in settings of intended use (likely at least up to 40°C and 90% relative humidity).	
4. Product configuration			
4.1. Service and support	None required.	If an instrument is required, its useful lifetime may be limited in number of use events or months / years of use (as indicated by Instructions for Use or internal instrument internal display) Initial lifetime calibration should be done at the time of manufacture, and/or instrument is automatically self-calibrating without user input.	
4.2. Waste disposal	Does not include material that cannot be disposed of in normal laboratory biohazard or general waste streams.	Ideally, consumables would be made from renewable or biodegradable materials.	
4.3. Labelling and instructions for use (IFUs)	Compliance required per stringent regulatory authority and WHO PQ guidance; Product Insert shall be available in relevant local language(s) and shall include Instructions for Use (IFUs) for the test.	WHO PQ label/IFU guidance should be applied, regardless of whether the test is WHO pre-qualified or not.	
5. Price and registration			
5.1. Target pricing per test	End-user price point of US$ 2 or less. Pricing shall warrant affordability in settings of need, without being a detriment to test performance.	Price points shown are for consumables only (i.e., no additional equipment costs associated with a lab-based test) and are guestimates provided by PPC development group; will require a detailed business/ROI case and COGS analyses for proposed designs.	

		Reduction of relapse episodes justifies a higher price point than what is recommended for an antigen *P. vivax* RDT.	
5.2. Capital cost, if applicable	Modest (≤ US$ 2000) to zero, based on purchase commitment.	If applicable, preference to use an existing standard platform as opposed to a single-use laboratory platform.	
5.3. Product registration (i.e., substantiation to regulatory body of product claims)	Registration required for export from country of origin • WHO PQ if/ once pathway is established • Country-level registration (if required/ applicable for target countries)		

PPC 2. Laboratory based test for risk of *P. vivax* relapse

Characteristics		Background	Additional notes
1. General requirements			
1.1. Intended use	Test to identify communities or individuals at risk of Plasmodium vivax (*P. vivax*) relapse based on detection of analyte(s) indicative of *P. vivax* hypnozoite carriage and/ or current sequestered infection and/ or recent (blood borne) infection with *P. vivax*. Unlike the POC test (see PPC #1) this test will be used to screen large numbers of individuals simultaneously as part of surveillance and/or monitoring activities related to *P. vivax* control and elimination.	Emerging data shows *P. vivax* parasites sequestered in sites of erythropoiesis (bone marrow, spleen and liver) in infectious individuals who may carry a high relapse risk.[12,13] "Recent infection" could refer to a previous 9 month window, based on available data from human infection studies and epidemiological studies.[9] The test is designed for being conducted as a centralized laboratory-based test, and could be used in the context of a "screen and treat" application. The test allows for collecting and transporting samples to a central lab for final analysis and is suitable for medium to high-throughput testing.	
1.2. Targeted population	All individuals living in *P. vivax* endemic settings where the transmission level ranges from low to elimination, and who are suspected of having latent or sequestered *P. vivax* infection.	A "screen and treat" approach may be applied for individuals identified to be at risk of *P. vivax* relapse, if follow up is feasible in the context of use. If the test is positive then it may inform radical cure treatment in line with WHO or national treatment guidelines for acute *P. vivax* infection.	
1.3. Lowest infrastructure level	Test use features allow for application in "limited infrastructure" conditions (Level 1); test use may require electricity and cold chain for transport and storage of reagents.	See Annex 1: Definition of health system infrastructure levels.	
1.4. Lowest level user	The test may require trained laboratory professionals.		

1.5. Test training requirements	≤2 days of training to perform the test.		
2. Design			
2.1. Portability	No restrictions on portability, but should be feasible to ensure safe and practical delivery and set-up to intended-use laboratory settings.	Use in mobile laboratories could be envisioned, and would be an ideal characteristic in terms of expanding its usability.	
2.2. Power requirements	Mains power (Local 100–240 V alternating current (AC), 50 or 60 Hz mains power)	Ideally also compatible with direct-current (DC) local supplies such as solar and other renewable power sources or an external uninterruptable power supply (UPS).	
2.3. Maintenance and calibration	Periodic maintenance and calibration of any instrumentation should be minimized.	If specialized services are needed but not locally available, an alternative mechanism of timely support must be provided. Device self-monitoring /alerts on the need for calibration.	
2.4. Sample type / collection	Capillary blood from finger sticks and venous blood, or non-invasive/minimally-invasive biological samples collected according to routine procedures. Samples should be amenable to transport under ambient conditions and to short-term storage.	Non-invasive or minimally-invasive samples might include saliva, urine or volatile organic compounds etc.	
2.5. Sample preparation / Test procedure	Sample preparation steps should be limited in number and compatible with medium- to high-throughput use.		

2.6. Sample volume	A volume of sample that can be obtained in a non-invasive/minimally-invasive way in all age groups.	For example, in the case of finger-prick sample, an acceptable volume would be 25-50µL.	
2.7. Target analyte	Marker or combination of markers identifying individuals at risk of *P. vivax* relapse.	Risk of relapse may be determined through detection of analytes that indicate (directly and/or indirectly) hypnozoite carriage: 1. Direct evidence: Hypnozoites metabolic markers, or other direct indicator 2. Proxy indicator: Immunological biomarker profiles against *P. vivax* to reflect acute or recently cured blood stage infection (e.g. in the last nine months, comparable to observed relapse patterns). A multiplexed approach interrogating several markers may enhance clinical diagnostic performance. 3. Proxy indicator: Detection of acute sequestered *P. vivax* infection (e.g. spleen, bone marrow) not detectable by current antigen based RDTs or by microscopy Proxy indicator 3 may be insufficiently sensitive and may need to be combined with evidence from 1 or 2.	
2.8. Type of analysis and detection	Quantitative or semi-quantitative analysis generating signal outputs which may be detectable by an instrument.	Choice and benefits of quantitative vs. semi-quantitative type of analysis may depend on the exact nature of the targets measured.	
2.9. Result output	Results may be quantitative or semi-quantitative but should allow for actionable outputs such as relapse risk stratification.	Same as above.	

2.10. Quality Control	Built in process control indicator (test control) and positive analyte controls could be provided to allow users to perform quality controls.		
2.11. Supplies needed	Reagents and supplies included in kit associated with minimal import restrictions.	Preferably animal-free, no BSA, and no triton X-100, for example.	
2.12. Safety	Safe to both the patient and user. Does not expose them to any unnecessary risks. Normal use does not create any additional hazards to the operator when observing Universal Blood Safety precautions.	For example, in the case of finger-stick sampling, an auto-retracting sterile lancet should be used.	
3. Performance			
3.1. Species differentiation	Ideally detects *P. vivax* only.	Interference with other Plasmodium species may be acceptable given the low *P. vivax*-endemicity of the intended-use settings, and given that mixed-species infections may be present.	
3.2. Diagnostic / clinical sensitivity	> 80% sensitivity to detect a future relapse event.	Test performance may be expected to out-perform the PPC#1 POC test given higher investment costs, but as test results are primarily to inform population-level risk levels, rather than individual treatment decisions (though both uses could be envisaged), less stringent performance may be acceptable. The reference is the occurrence of a *P. vivax* relapse in the months following testing. Successfully detecting all blood-stage relapses is challenging and a high-sensitivity assay (e.g. PCR) should be used to repeatedly test individuals with high regularity. Well-characterized biobanked samples with	Results from recent field studies and modelled impact scenarios assess trade-off between over-treatment with different mass-test and treat strategies and MDA in terms of diagnostic sensitivity and specificity.[22,23] WHO encourages development of open biobanks that would facilitate

		accompanying metadata (clinical histories and infection status by PCR) could be used to assess diagnostic performance.	R&D for tests designed to meet PPC targets.
3.3. Diagnostic / clinical specificity	> 90% specificity to detect a future relapse event.	Same comments as above	Same comments as above
3.4. Time to results	<4 hours to developed test result for a batch run of > 90 specimens, including sample preparation time.	Time to result is less critical, but operator time spent on a batch run should be minimized.	
3.5. Result stability	Test outcome (per batch run) shall be automatically stored in device, for > 1000 tests.	Ability to interpret and store final test results in a computer-aided manner not constrained by timed steps helps greatly in resource-constrained settings.	
3.6. Throughput	Minimum capacity of >90 specimens per batch run (can be run with less), and ability to run two batches per 8 hours.	Must also be able to label / track individual specimens from accession to results.	
3.7. Target shelf life / storage conditions	≥18 months, 15°C - 35°C, 75% RH, but acceptable if reagents not needed for sample collection require storage at 4°C to neg. 20°C; reagents need to withstand temperature excursion to 45°C for two weeks without effect on shelf life.	Requirements relate to test kits that are used in the field, including specimen collection. NOTE: consumables required for laboratory-based testing procedures may or may not require cold chain.	
3.8. Ease of use	Five or fewer timed steps; ≤15 user steps, instructions for use should include diagram of method and results interpretation.		
3.9. Ease of results interpretation	Results can be interpreted by a suitable instrument.		

3.10. Operating temperature	15°C to 35°C; if in-device temperature compensation is required, it will be automatic.		
4. Product configuration			
4.1. Service and support	Maintenance / technical support must be available from manufacturer for the region of use (equipment and/or procedures).		
4.2. Waste disposal	Does not include material that cannot be disposed of in normal laboratory biohazard waste streams.	Ideally, consumables would be made from renewable or biodegradable materials.	
4.3. Labelling and instructions for use (IFUs)	Compliance required per stringent regulatory authority and WHO PQ guidance; Product Insert shall be available in relevant local language(s) and shall include Instructions for Use (IFUs) for the test	WHO PQ label/IFU guidance should be applied, regardless of whether test is WHO pre-qualified or not.	
5. Price and registration			
5.1. Target pricing per test	End-user price point of US$ 2 or less. Pricing shall warrant affordability in settings of need, without being a detriment to test performance.	Price points shown are for *consumables only* (i.e., no additional equipment costs associated with a lab-based test) and are guestimates provided by PPC development group; a detailed business/ROI case and COGS analyses will be required for proposed designs.	
5.2. Capital cost	Multi-use/standard platform pricing should aim for < US$ 15 000.	Use a standard platform as opposed to a single-use laboratory platform may constitute an advantage to the user.	
5.3. Product registration (i.e., substantiation to	Registration required for export from country of origin (e.g., KFDA)		

regulatory body of product claims)	• WHO PQ if/ once pathway is established • Country-level registration (if required/ applicable for target countries)		

Annex 1. Definition of health system infrastructure levels

Table A1 outlines the definition of health system infrastructure levels, as described in Ghani et al[iii] and the Maputo Declaration.[iv]

Table A1. Definition of health infrastructure levels

Characteristics	Level 0	Level 1	Level 2	Levels 3 and 4
Description	In the community or home	Lowest level of health care system with a laboratory	First level of referral health care and laboratories	Second and higher levels of referral health care and laboratories
Examples of locations	In homes, health fairs, health posts, clinics with no laboratory, pharmacies	Health centres (Africa), rural health centres (Asia and Latin America)	Hospitals (Africa), urban health clinics (Asia and Latin America), clinical laboratories in the developed world	Hospitals (Latin America and Asia), national clinical/reference laboratories (Africa), surveillance laboratories, research laboratories
Electricity	Not reliably available	Not reliably available	Available, expected to have refrigeration	Available
Clean water	Not reliably available	Not reliably available	Available	Available
Physical and laboratory infrastructure and laboratory equipment	No laboratory	Not all facilities have laboratories. If present, minimally equipped (e.g. microscope, centrifuge) or moderately equipped (see level 2 description laboratories)	Moderately equipped laboratories (e.g., additional equipment for basic chemistry and manual immunoassays)	Well-equipped laboratories (e.g., automated and advanced equipment)
Personnel	Community health care workers, nurses, family members, pharmacists, traditional medicine practitioners	Nurses, sometimes physicians, laboratorians with a range of training	Nurses, physicians, moderately and well-trained laboratorians	Nurses, physicians, well-trained laboratorians

[iii] Ghani AC, Burgess DH, Reynolds A, Rousseau C. Expanding the role of diagnostic and prognostic tools for infectious diseases in resource-poor settings. Nature. 2015;528:S50–52. doi: 10.1038/ nature16038.

[iv] The Maputo Declaration on strengthening of laboratory systems. Brazzaville: World Health Organization Regional Office for Africa; 2008 (https://www.who.int/publications/m/item/themaputo-declaration-on-strengthening-of-laboratory-systems, accessed 6 June 2022).

References

[1] WHO. Global technical strategy for malaria 2016-2030 (GTS). Geneva: World Health Organization, 2015a. https://www.who.int/publications/i/item/9789241564991

[2] WHO. World Malaria Report 2022. Geneva: World Health Organization, 2022. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2021

[3] Mahittikorn, A., Masangkay, F.R., Kotepui, K.U. *et al.* Comparison of *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri* infections by a meta-analysis approach. *Sci Rep* 11, 6409 (2021). https://doi.org/10.1038/s41598-021-85398-w

[4] WHO. Malaria eradication: benefits, future scenarios & feasibility. Geneva: World Health Organization, 2020. https://www.who.int/publications/i/item/9789240003675

[5] Robinson LJ, Wampfler R, Betuela I, et al. Strategies for understanding and reducing the *Plasmodium vivax* and *Plasmodium ovale* hypnozoite reservoir in Papua New Guinean children: a randomized placebo-controlled trial and mathematical model. *PLoS Med.* 2015; 12(10): e1001891.

[6] Commons RJ, Simpson JA, Watson J, et al. Estimating the proportion of *Plasmodium vivax* recurrences caused by relapse: a systematic review and meta-analysis. *Am J Trop Med Hyg*. 2020; 103(3):1094-1099.

[7] Douglas NM, Anstey NM, Buffet PA, et al. The anaemia of *Plasmodium vivax* malaria. *Malar J*. 2012; 11:135

[8] Dini S, Douglas NM, Poespoprodjo JR, et al. The risk of morbidity and mortality following recurrent malaria in Papua, Indonesia: a retrospective cohort study. *BMC Med*. 2020; 18:28

[9] White N.J. Determinants of relapse periodicity in *Plasmodium vivax* malaria. *Malar J.* 2011; https://doi.org/10.1186/1475-2875-10-297

[10] Commons RJ, Simpson JA, Thriemer K, et al. Risk of *Plasmodium vivax* parasitaemia after *Plasmodium falciparum* infection: a systematic review and meta-analysis. *Lancet Infect Dis.* 2019; 19:91-101.

[11] WHO. Confronting *Plasmodium vivax* malaria. Geneva: World Health Organization, 2015b. https://apps.who.int/iris/handle/10665/181163

[12] Kho S, Qotrunnada L, Leonardo L, et al. Evaluation of splenic accumulation and colocalization of immature reticulocytes and *Plasmodium vivax* in asymptomatic malaria: A prospective human splenectomy study. *PLoS Med*. 2021; 18(5):e1003632

[13] Kho S, Qotrunnada L, Leonardo L, et al. Correspondence: Hidden biomass of intact malaria parasites in the human spleen. *N Engl J Med*. 2021; 384:2067-2069.

[14] Brito MAM, Baro B, Raiol TC, et al. Morphological and transcriptional changes in human bone marrow during natural *Plasmodium vivax* malaria infections. *J Infect Dis*. 2022; 225(7):1274-1283.

[15] Global Malaria Programme, Case management, Diagnosis, Rapid diagnostic tests. Geneva: World Health Organization. https://www.who.int/teams/global-malaria-programme/case-management/diagnosis/rapid-diagnostic-tests

[16] Cunningham J, Jones S, Gatton ML. *et al.* A review of the WHO malaria rapid diagnostic test product testing programme (2008–2018): performance, procurement and policy. *Malar J.* 2019; **18**, 387.

[17] Cheng Q, Cunningham J, Gatton ML. Systematic review of sub-microscopic *P. vivax* infections: prevalence and determining factors. *PLoS Negl Trop Dis*. 2015; 9(1), e3413.

[18] Anstey NM, Douglas NM, Poespoprodjo JR, Price RN. *Plasmodium vivax*: clinical spectrum, risk factors and pathogenesis. *Adv Parasit*. 2012; *80*, 151–201.

[19] Agarwal R, Choi L, Johnson S, Takwoingi Y. Rapid diagnostic tests for *Plasmodium vivax* malaria in endemic countries. *Cochrane Database Syst Rev*. 2020; 11: CD013218.

[20] Gualdron-Lopez M, Flannery EL, Kangwanrangsan N, et al. 2018. Characterization of *Plasmodium vivax* proteins in plasma-derived exosomes from malaria-infected liver-chimeric humanized mice. *Front Microbiol*. 2018; 9:1271.

[21] Howes RE, Gregg A, Harbers M, et al. Developing a serological rapid test for an unmet diagnostic niche: the *Plasmodium vivax* hypnozoite. *American Society of Tropical Medicine & Hygiene annual meeting*. 2020; Virtual meeting; Presentation 1641.

[22] Longley RJ, White MT, Takashima E, et al. Development and validation of serological markers for detecting recent *Plasmodium vivax* infection. *Nature Med*. 2020; 26:741-9.

[23] Noviyanti R, et al. Identifying future *Plasmodium vivax* relapses with serological markers of exposure in a returning Indonesian soldier cohort. in *American Society of Tropical Medicine & Hygiene.* 2021; Virtual Meeting; Abstract 0886.