



Testing for G6PD deficiency for safe use of primaquine in radical cure of *P. vivax* and *P. ovale* malaria

Policy brief

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BACKGROUND

Primaquine is currently the only medicine for treating relapses of *Plasmodium vivax* and *P. ovale* malaria, due to its specific activity against malaria hypnozoites. Despite a reduced sensitivity of these parasites in some countries, requiring increased doses, primaquine has remained highly effective for anti-relapse therapy since its introduction in 1952. The full potential of this medicine to prevent relapses and reduce the transmission of vivax malaria has however not been fully used, owing to concerns about its safety. The medicine induces dose-dependent acute haemolytic anaemia in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency, a genetically X-linked disorder. This condition is widely prevalent affecting over 350 million people globally, with a prevalence of 3–35% in tropical areas.

The recommended dosage for safe use of primaquine must therefore be adapted to the G6PD status of the patient. This status is, however, rarely known, except in the few countries (e.g. Malaysia and the Philippines) in which G6PD testing is part of newborn screening programmes. Often, G6PD testing is not available at points of care after a diagnosis of *P. vivax* or *P. ovale* malaria. As a result, primaquine is usually given without prior G6PD testing – thus exposing some patients to the risk for haemolytic anaemia – or is not administered – exposing patients to the risk for repeated relapses of *P. vivax* malaria, with consequent morbidity and transmission.

When new point-of-care G6PD tests became available, WHO convened in October 2014 a group to review the evidence from recent evaluations of the performance of testing devices appropriate for use in tropical and resource-limited settings (1). The conclusions were reviewed by the Malaria Policy Advisory Committee and by the Technical Expert Group on Malaria Chemotherapy for inclusion in the third edition of the WHO Guidelines for the treatment of malaria (2).

The aim of this policy brief is to disseminate the new WHO recommendations on G6PD testing to ensure safe administration of primaquine for preventing relapse of *P. vivax* and *P. ovale* malaria. It offers national malaria control programmes guiding principles and practical advice on:

- classification and testing of G6PD deficiency, highlighting the differences between males and females;
- a diagnostic and treatment algorithm for qualitative point-of-care G6PD testing and anti-relapse treatment with primaquine;
- tables for dosing primaquine and information on the sourcing of quality-assured primaquine;
- checklists for counselling patients, recognizing the symptoms of acute haemolytic anaemia and managing side-effects;
- risk-benefit assessments before administering primaquine without G6PD testing, including considerations for G6PD testing in specific countries and geographical areas;
- quantitative and qualitative G6PD tests and the preferred product characteristics of qualitative G6PD tests for use at points of care and
- planning and conducting G6PD testing at country level.



WHO RECOMMENDATION

The G6PD status of patients should be used to guide administration of primaquine for preventing relapse.

- To prevent relapse, treat *P. vivax* or *P. ovale* malaria children and adults (except pregnant women, infants aged < 6 months, women breastfeeding infants aged < 6 months, women breastfeeding older infants unless they are known not to be G6PD deficient and people with G6PD deficiency) with a 14-day course of primaquine at 0.25–0.5 mg base/kg body weight daily in all transmission settings.
- In people with G6PD deficiency, consider preventing relapse by giving primaquine at 0.75 mg base/kg body weight once a week for 8 weeks, with close medical supervision for potential primaquine-induced haemolysis.
- When a patient's G6PD status is unknown and G6PD testing is not available, a decision to prescribe primaquine must be based on an assessment of the risks and benefits of adding primaquine.
- For women who are pregnant or breastfeeding, consider weekly chemoprophylaxis with chloroquine until delivery and breastfeeding are completed; then, on the basis of the woman's G6PD status, treat with primaquine to prevent future relapse (2).

EXPECTED BENEFITS

Complete treatment of *P. vivax* and *P. ovale* malaria requires treatment of both blood-stage infections (to achieve immediate clinical cure and thus avoid progression to severe disease) and liver-stage infections (to prevent future relapses and avoid onward transmission). Wide-scale implementation of the new WHO recommendations on safe administration of primaquine for preventing relapses is expected to have a positive impact for both individuals and public health.

- Safe administration of primaquine on the basis of the results of G6PD testing will reduce morbidity due to relapse in patients with *P. vivax* or *P. ovale* malaria. At the same time, wider G6PD testing will reduce the risk of G6PD-deficient patients for acute haemolytic anaemia.
- Besides these advantages for individual patients, health systems will benefit from fewer cases of malaria relapse and of acute haemolytic anaemia, decreasing the demand for management of these conditions, including the burden on blood transfusion services.
- In addition, widespread G6PD testing and subsequent safe administration of primaquine will reduce the rates of relapse of *P. vivax* and *P. ovale* malaria and thus contribute to reducing transmission of these parasites. The impact will be stronger on infections with tropical strains of *P. vivax*, which are associated with higher relapse rates.

PRIMAQUINE AND G6PD DEFICIENCY

Primaquine

Primaquine, an 8-aminoquinoline derivative, has been used since the early 1950s for both radical treatment of *P. vivax* and *P. ovale* malaria and as a gametocytocide in *P. falciparum* malaria (3). In *P. falciparum* malaria, the compound is highly effective in reducing the transmissibility of gametocytes when given at a single low dose of 0.25 mg base/kg body weight. At this dose, primaquine is well tolerated, regardless of the patient's G6PD status. For elimination of *P. vivax* and *P. ovale* liver-stage infections (radical cure), primaquine must be given at a higher dose, 0.25–0.50 mg base/kg body weight daily for 14 days, in addition to the antimalarial medicine that cures the blood-stage infection. In G6PD-normal patients, this dose of primaquine is remarkably safe, well tolerated and highly efficacious in preventing relapse. In a significant proportion of G6PD-deficient patients, however, the 14-day regimen of primaquine induces dose-dependent, potentially severe haemolysis.

G6PD deficiency: prevalence, genotypes and phenotypes

G6PD is a critical housekeeping enzyme in red blood cells that intervenes against oxidative challenge by producing the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). Erythrocytes have no alternative pathways for G6PD-dependent NADPH production. G6PD deficiency is an inherited sex-linked genetic disorder, which is associated with some protection against severe *P. falciparum* malaria infections but also with increased susceptibility to oxidant haemolysis. The distribution of G6PD deficiency is shown in Figure 1 and that of *P. vivax* malaria in Figure 2.

FIGURE 1

Prevalence of G6PD deficiency

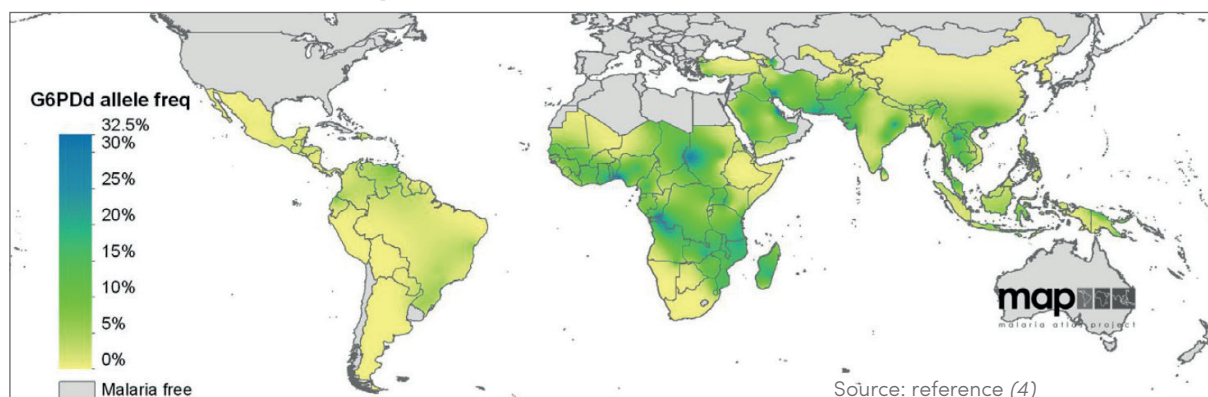
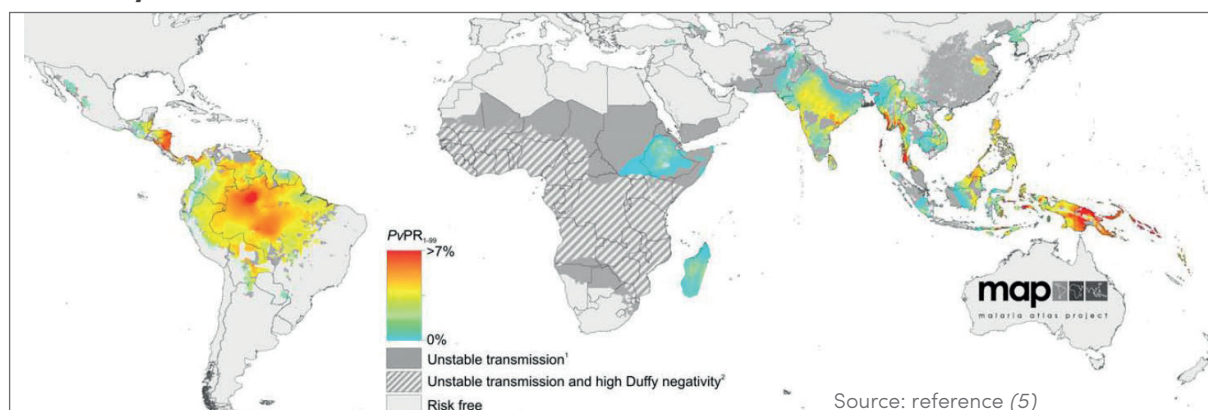


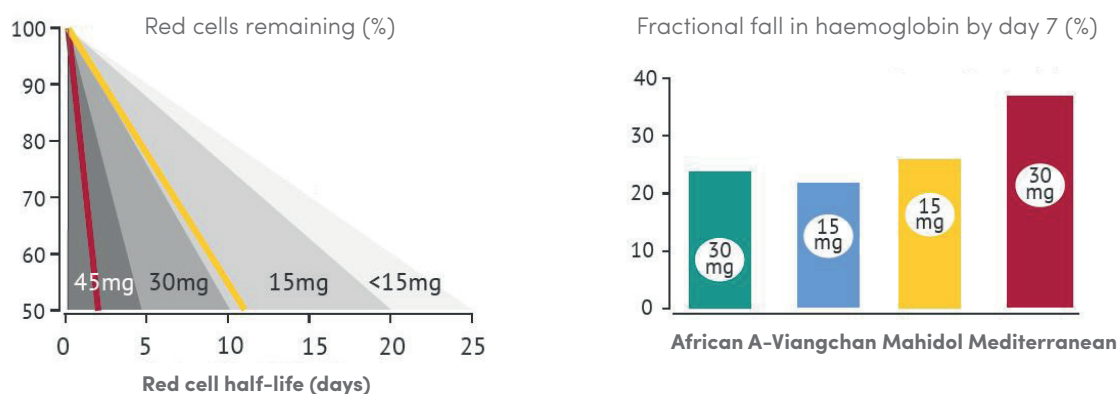
FIGURE 2

Endemicity of *P. vivax* in 2010



More than 180 different genetic variants of G6PD deficiency are known. Nearly all the variants make the red blood cells susceptible to oxidant haemolysis, but the severity of haemolytic anaemia depends on both the dose and the frequency of administration of primaquine and the variant of the G6PD enzyme. Two of the most prevalent variants represent the two ends of the severity spectrum. The Mediterranean variant, which is the main one found in Europe, west and central Asia and northern India, is among the most severe, and the African A- variant, found in sub-Saharan Africa and in African-Americans, is among the mildest.¹ In people with less severe G6PD variants, primaquine-induced haemolysis usually becomes evident after 1 or 2 days of treatment, when the oxidant defences of all the older erythrocytes have been depleted (7–9). If treatment with primaquine is continued in people with the African A- variant, haemolysis lessens, and the haemoglobin concentration starts to rise again, despite further administration of the medicine, as reticulocytes enter the circulation to replace the haemolysed cells. These young red cells contain five times more G6PD than the oldest red cells and are therefore relatively resistant to the haemolytic effect. Further haemolysis occurs, however, with higher doses (9). In contrast, in people with the Mediterranean variant, haemolysis continues if primaquine is not stopped, and life-threatening anaemia may result. Figure 3 illustrates the survival of red blood cells and the extent of anaemia after daily administration of primaquine in people with different variants of G6PD deficiency. The left hand graph shows red cell survival after different daily doses of primaquine in adults with the African A- variant of G6PD deficiency: the higher the daily dose of primaquine, the shorter the red cell half-life. The right graph displays the average fractional fall in haemoglobin concentration by day 7 (time of the usual nadir) with daily doses of primaquine in people with African A-, Viangchan, Mahidol and Mediterranean G6PD deficiency variants.

FIGURE 3
Red cell survival and degree of anaemia following daily primaquine in different G6PD deficiency variants



As G6PD deficiency is an X-linked disorder, males have only one G6PD allele, whereas females have two. Hence, there are two distinct G6PD genotypes in males (wild type and hemizygous) but three in females (wild type, homozygous and heterozygous). During embryonic development of females, one of the two X chromosomes in somatic cells becomes inactivated in an apparently random manner, and the active or inactive state of the X chromosome is maintained in the progeny of each cell. This phenomenon, known as lyonization, is responsible for the variable levels of enzymatic activity in heterozygous females, reflecting the proportion of red blood cells with inactivated G6PD enzyme. The five genotypes in males and females translate into

1. The recent finding of severe anaemia requiring blood transfusion in African children with the A- variant after exposure to chlorproguanil-dapsone-artesunate in randomized clinical trials has led to reconsideration of abandoning the term "mild-to-moderate" G6PD deficiency when referring to these variants. (6).

three phenotypes: G6PD normal and G6PD deficient in both males and females and G6PD intermediate (with an enzyme activity 30–80% of normal) in heterozygous females only.

Males



- **G6PD normal.** Any male with > 80% of normal² red blood cell G6PD activity is considered G6PD normal. It is presumed that he is hemizygous for a G6PD normal allele.
- **G6PD deficient.** Any male with < 10% of normal² red blood cell G6PD activity is regarded as G6PD deficient. It is presumed that he is hemizygous for a G6PD deficiency allele.

Females



- **G6PD normal.** Any female with > 80% of normal² red blood cell G6PD activity is considered G6PD normal. It is presumed that she is either homozygous for a G6PD normal allele or heterozygous for a G6PD deficiency allele and a G6PD normal allele, with a predominance of a G6PD normal² red blood cell population.
- **G6PD intermediate.** Any female with 30–80% of normal² G6PD activity is considered G6PD intermediate. It is presumed that she is heterozygous for a G6PD deficiency allele and a G6PD normal allele.
- **G6PD deficient.** Any female with < 30% of normal² red blood cell G6PD activity is considered G6PD deficient. It is presumed that she is either homozygous for a G6PD deficiency allele, bi-allelic or heterozygous for a G6PD deficiency allele, with predominance of a G6PD-deficient red blood cell population.

The phenotype ultimately decides on the primaquine sensitivity of a patient, i.e. the likelihood that the patient develops AHA after primaquine administration (Table 1).

TABLE 1

Relations between G6PD deficiency genotypes, enzyme activity and sensitivity to primaquine

GENOTYPE	SEX	G6PD ENZYME ACTIVITY*	PHENOTYPE	SENSITIVE TO PRIMAQUINE
XY – wild type	Male	Normal	Normal	No
X*Y – hemizygote		< 30% of normal	Deficient	Yes
XX – wild type	Female	Normal	Normal	No
X*X* – homozygote		< 30% of normal	Deficient	Yes
X*X – heterozygote		< 30% of normal	Deficient	Yes
X*X – heterozygote		Between 30% and 80% of normal	Intermediate	Possible
X*X – heterozygote		> 80% of normal	Normal	Unlikely

* Levels of a G6PD activity in the blood-cell population above 30% of normal values are considered to confer an acceptable risk with normal therapeutic doses of primaquine in G6PD normal patients. This value is based on the detection parameters of the nicotinamide adenine dinucleotide phosphate (NADPH) fluorescent spot test, which has been extensively used in operational settings, both in guiding treatment decisions and to guide which patients should be included in clinical trials (10).

2. The normal G6PD value in a population is best defined in the male sub-population as males can only be normal or deficient in G6PD. In order to remove bias from G6PD deficient males, G6PD deficient males are excluded from the population of males from which the median or mean is used to define the study population normal (100% G6PD activity). One approach to determining this value is described in Domingo et al. Malaria Journal 2013, 12:391.



POINT-OF-CARE TESTING FOR G6PD DEFICIENCY IN MALES AND FEMALES

Only few qualitative point-of-care G6PD tests are currently on the market. In most, the threshold for detecting G6PD enzyme activity is 30%; with an enzyme activity > 30% being considered “G6PD normal” and activity < 30% being considered “G6PD deficient”. This is relevant for daily administration of primaquine, as development of acute haemolytic anaemia is expected in individuals with red cell G6PD activity < 30% of the normal (2) (see Table 1).



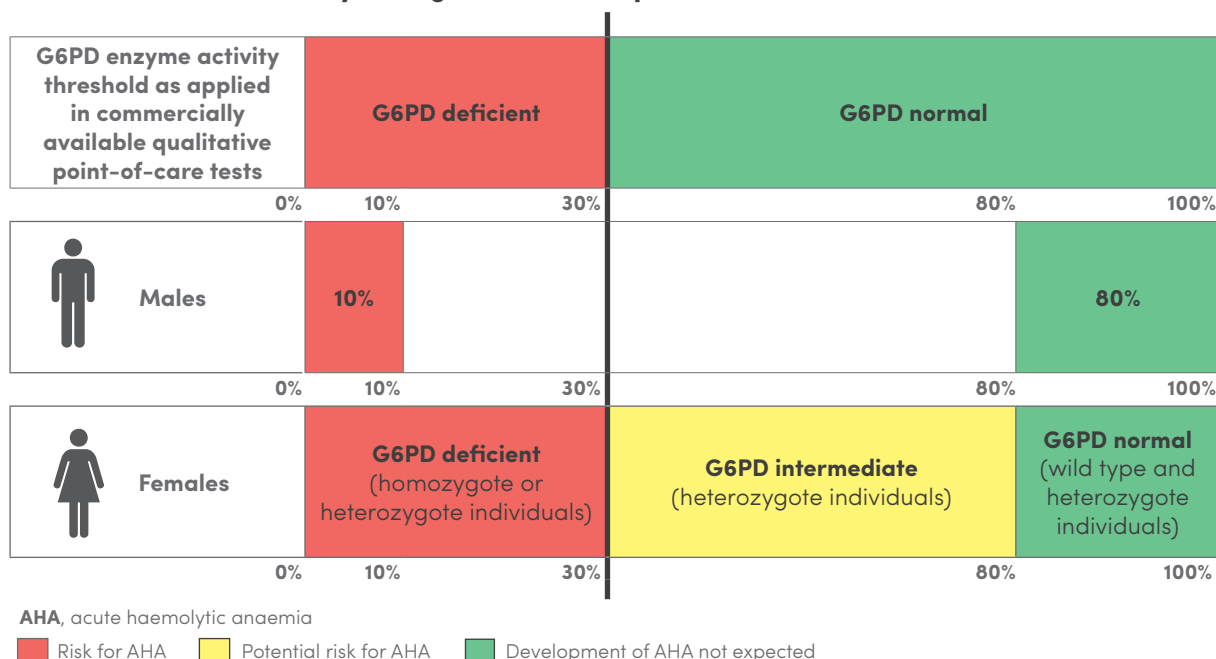
- **Males.** Classification of male patients as G6PD normal or G6PD deficient with these tests is based on the straightforward application of the 30% threshold, as males have either normal G6PD enzyme activity (wild type), typically higher than 80%, or a remaining enzyme activity < 10% of normal (hemizygote). Interpretation of the test result is unambiguous and allows determination of the dose and frequency for safe administration of primaquine to male patients.



- **Females.** Use of these tests is, however, more complex for female patients, as they may (rarely) have < 30% of enzyme activity or (more often) intermediate G6PD enzyme activity, between 30% and normal values. A proportion of female heterozygotes with intermediate G6PD enzyme activity may experience haemolysis after daily administration of primaquine; however, they cannot be identified unambiguously with the currently available point-of-care G6PD tests, which classify the range of 30–80% “intermediate” activity as “G6PD normal”. At present, patients with “G6PD intermediate” enzyme activity can be classified only by quantitative testing methods, which are not yet available at points of care in rural tropical settings.

Figure 4 illustrates the relation between the results of qualitative point-of-care G6PD tests and enzyme activity in males and females on the basis of the 30% activity threshold detected in these tests.

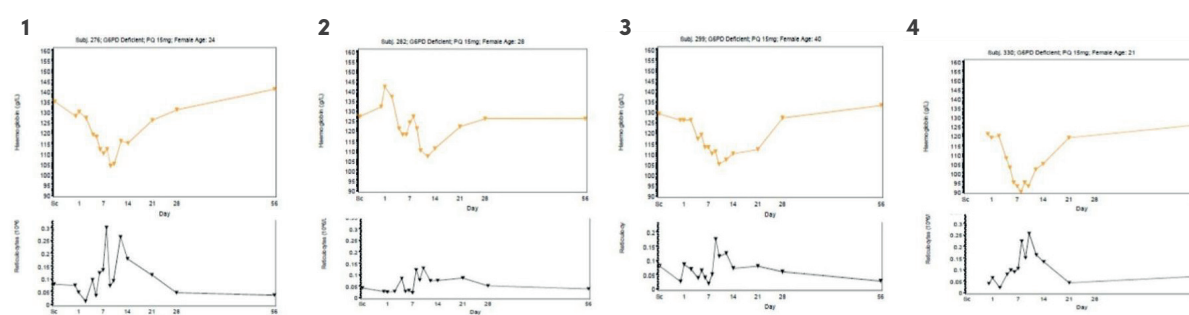
FIGURE 4
Qualitative G6PD deficiency testing with available point-of-care tests in males and females



ALGORITHM FOR QUALITATIVE TESTING FOR G6PD DEFICIENCY AT POINTS OF CARE AND SAFE ADMINISTRATION OF PRIMAQUINE TO PREVENT RELAPSE OF *P. VIVAX* AND *P. OVALE* MALARIA IN MALE AND FEMALE PATIENTS

In non-G6PD-deficient individuals, a 14-day regimen of primaquine has been shown to be safe and effective for radical treatment of *P. vivax* and *P. ovale* malaria (2).³ Evidence from a few studies, however, indicates a substantial haemolytic response in heterozygous females with G6PD deficiency (11, 12). In a recent study of tafenoquine, an 8-aminoquinoline derivative under development, sponsored by GlaxoSmithKline, four heterozygous women who were treated with 15 mg primaquine base for 14 days showed a pattern and a decrease in haemoglobin (2.5 g/dL) similar to those observed in all patients with G6PD deficiency. The G6PD activity in these women was 40–60% of normal.⁴ Figure 5 shows the individual haematological parameters. These findings in women with the Mahidol variant effectively mirror those in the experiments described below in African A- hemizygous men.

FIGURE 5
Change in haemoglobin levels after exposure to daily primaquine for 14 days at 0.25 mg/kg/day in four women heterozygous for G6PD deficiency



Source: GlaxoSmithKline, personal communication

Orange line, haemoglobin; black line, reticulocytes

The above figures demonstrate a substantial hemolytic response to daily primaquine at 0.25 mg/kg in heterozygous females with G6PD deficiency. The haemoglobin nadir occurs between days 8 and 11, representing drops in haemoglobin ranging from 17% to 25%; thereafter the haemoglobin recovers to pre-treatment levels despite continued primaquine dosing up to day 14. As shown in all graphs, the spike in reticulocytosis following the AHA commences at about day 7 of dosing.

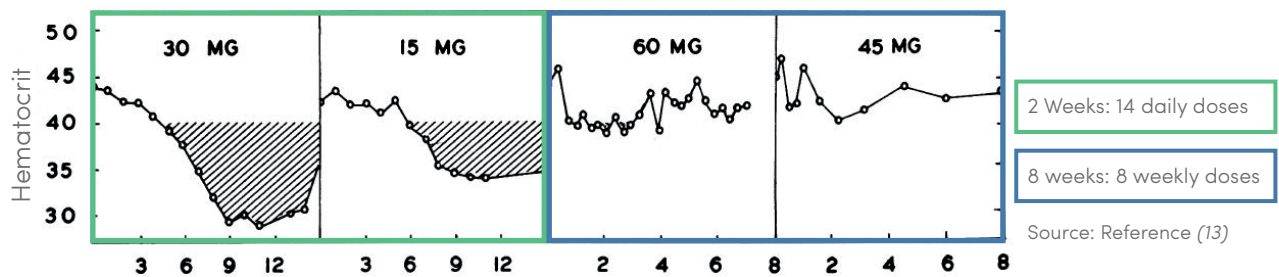
The severity of oxidant haemolysis in heterozygous females varies from that observed in hemizygous males (if the majority of their red cells are G6PD deficient) to very little haemolysis (if the majority of their red cells are G6PD normal) (2).

To reduce the risk for haemolysis of individuals who do not have severe variants of G6PD deficiency, an intermittent primaquine regimen of 0.75 mg base/kg body weight weekly for 8 weeks can be given, under medical supervision. This regimen was safe and effective in people with the African A- genotype (13). Figure 6 shows the extent of haemolysis after different primaquine regimens in the same person, who was known to be sensitive to 30 mg primaquine daily. Weekly administration reduced the anaemia by allowing haematological recovery after each dose.

3. Primaquine crosses the placenta and may cause haemolysis in a G6PD-deficient fetus; it is therefore not recommended for use during pregnancy or during breastfeeding unless the G6PD status of the infant is known. Use of primaquine in infants < 6 months is not advised because of lack of data on its safety.
4. J. Green, personal communication.

FIGURE 6

Haemolytic response of the same person after daily and after weekly administration of primaquine



The risk for severe haemolysis is virtually restricted to G6PD-deficient individuals, which is why testing is so important (14). WHO recommendations for G6PD diagnosis and radical cure of *P. vivax* and *P. ovale* infections are compiled in below algorithm, which takes into consideration the detection limits of the available qualitative point-of-care G6PD tests (Figure 7).

Treatment options for male and female malaria patients according to their G6PD status

Note: If a G6PD test is undertaken, the result should be recorded. The WHO treatment recommendations based on results of qualitative G6PD tests are given below.

Males



- **G6PD normal** male patients (G6PD activity > 30%) should be treated with a 14-day course of primaquine at 0.25–0.5 mg base/kg body weight daily in all transmission settings.
- **G6PD deficient** male patients (G6PD activity < 30%) can be treated with primaquine at a dose of 0.75 mg base/kg body weight once a week for 8 weeks. The decision to give primaquine should depend on whether the treatment can be given under close medical supervision, with ready access to health facilities with blood transfusion services and patient counselling.

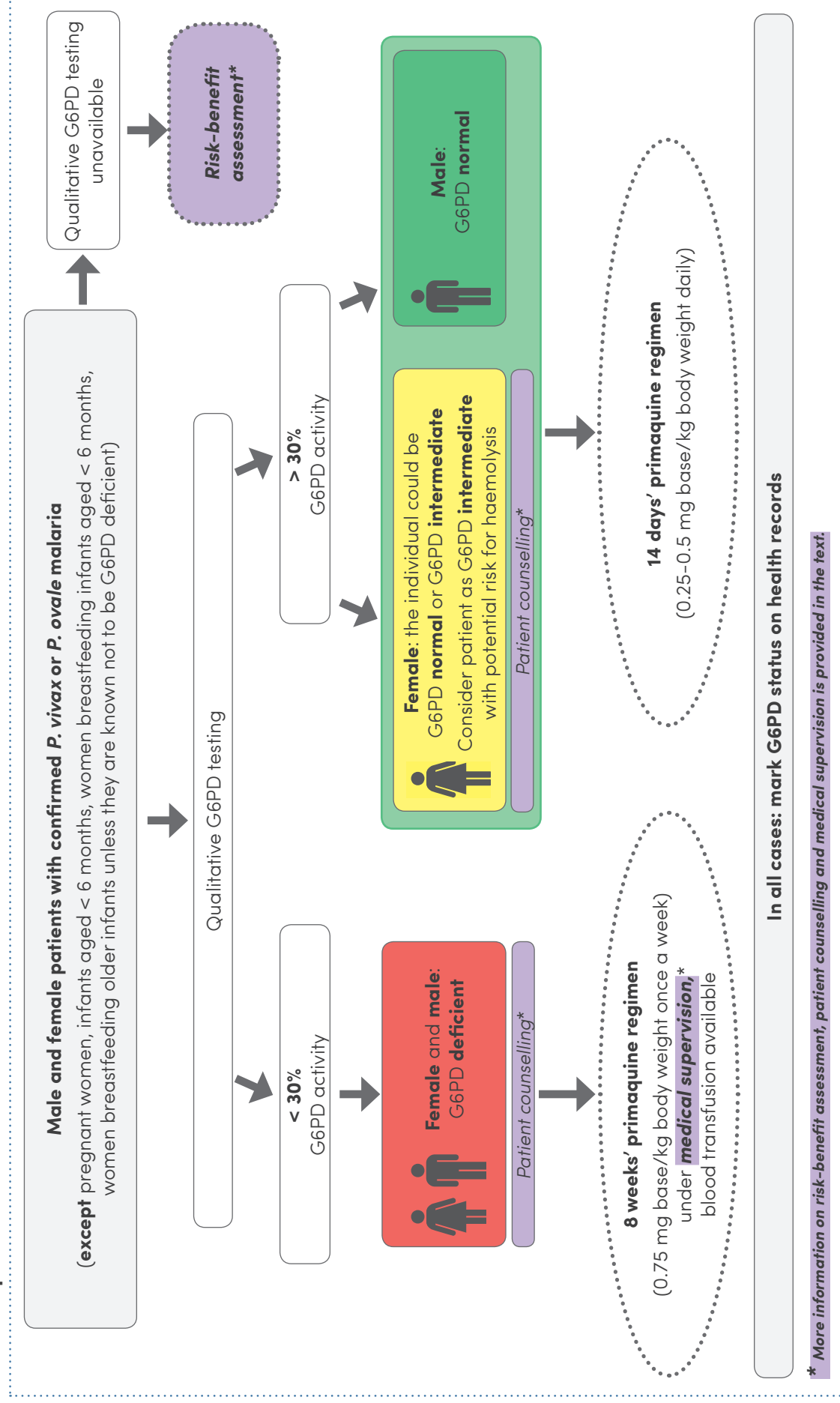
Females



- **G6PD normal** and **G6PD intermediate** female patients (both with G6PD activity > 30%, see Figure 4). Some heterozygote females who test as normal or not deficient in qualitative G6PD screening tests have intermediate G6PD activity and can still undergo substantial haemolysis. Intermediate deficiency (30–80% of normal) and normal enzyme activity (> 80% of normal) can be differentiated only in a quantitative test. In the absence of quantitative tests, all females should be considered as potentially having intermediate G6PD activity and given the 14-day regimen of primaquine, with counselling on recognizing symptoms and signs of haemolytic anaemia. They should be advised to stop primaquine and be told where to seek care should such signs develop.
- **G6PD deficient** female patients (G6PD activity < 30%) are either homozygous or heterozygous, with full expression of G6PD deficiency. This patient group can be treated the same way as G6PD deficient male patients, i.e. with primaquine at a dose of 0.75 mg base/kg body weight once a week for 8 weeks. The decision to give primaquine should depend on whether the treatment can be given under close medical supervision, with ready access to health facilities with blood transfusion services and patient counselling.

FIGURE 7

Algorithm for qualitative point-of-care testing and safe administration of primaquine to prevent relapse of *P. vivax* or *P. ovale* malaria in male and female patients



Dosage, administration and sourcing of quality-assured primaquine

Use of primaquine is recommended in all transmission settings (2). In general, primaquine is well tolerated at single doses of ≤ 0.5 mg base/kg body weight. Dosing is limited by abdominal discomfort at doses > 1 mg/kg body weight and when primaquine is taken on an empty stomach. Tolerability can be improved by taking the medicine with food (2).

Global guidance on dose calculations according to the therapeutic range of primaquine and the patient's body weight are presented in Table 2. These calculations might be adapted and refined according to quality representative national data with good age-weight correlations. At present, only 7.5 mg base and 15.0 mg base⁵ primaquine tablets are available commercially at internationally agreed quality standards. These tablets are small, not scored and difficult to cut.

TABLE 2
Primaquine dose calculation as per patient's body weight

PATIENT'S BODY WEIGHT (KG)	TARGET DOSE: 0.25 MG BASE / KG BODY WEIGHT (FOR TEMPERATE <i>P. VIVAX</i> STRAINS)	TARGET DOSE: 0.5 MG BASE / KG BODY WEIGHT (FOR TROPICAL, FREQUENTLY RELAPSING <i>P. VIVAX</i> STRAINS PREVALENT IN EAST ASIA AND OCEANIA)	TARGET DOSE: 0.75 MG BASE / KG BODY WEIGHT
	DOSE FREQUENCY: DAILY PRIMAQUINE DOSE FOR 14 DAYS (MG BASE)		DOSE FREQUENCY: WEEKLY PRIMAQUINE DOSE FOR 8 WEEKS (MG BASE)
10 – < 25	3.75	7.5	7.5 – 15.0
25 – < 50	7.5	15.0	30.0
50 – 100	15.0	30.0	45.0

The full 14-day or 8-week treatment course must be adhered to for full prevention of relapse. Methods to improve adherence include patient counselling, better packaging and directly observed treatment, in which each dose is given by a trained health worker. Patients should be advised of the possible risks and told that they should stop the medicine if they become ill or if their urine is dark or becomes black (10).

Only primaquine of proven quality that conforms to international standards should be used. At present, two products are approved by stringent regulatory authorities. Both are on the current Global Fund list of malaria pharmaceutical products (classified according to the Global Fund Quality Assurance Policy), which can be accessed at: http://www.theglobalfund.org/documents/psm/PSM_ProductsMALARIA_List_en/.

5. 7.5 mg primaquine base is equivalent to 13.2 mg primaquine phosphate; 15 mg primaquine base is equivalent to 26.3 mg primaquine phosphate.

Patient counselling and detection of primaquine-induced haemolysis

Primaquine is eliminated rapidly (3.5–8 hours), and haemolysis is self-limiting once administration of the medicine is stopped (2). Before being given primaquine, patients should be counselled according to the checklist in Box 1.

BOX 1

CHECKLIST FOR PATIENT COUNSELLING

Ideally with the help of adequate product information material (consider local languages, as required), patients should be informed about the following:

- ☐ Explain the benefit of primaquine administration.
- ☐ Enquire the patient for a medical history of haemolysis.
- ☐ Inform the patient about the risk for acute haemolytic anaemia when taking primaquine.
- ☐ Instruct the patient to monitor the colour of her or his urine.
- ☐ Instruct the patient to stop taking primaquine if her or his urine becomes dark.
- ☐ Inform the patient where to seek medical advice if her or his urine becomes dark (the nearest hospital with blood transfusion services).

Note: In some countries (e.g. Malaysia and the Philippines), screening of newborns for G6PD deficiency has been introduced, and the G6PD status is recorded on a personal medical card. Young children should be carefully monitored.

Health workers should be trained, with appropriate job aids, to recognize the symptoms of acute haemolytic anaemia and to determine when to refer patients for further assessment. A checklist for identifying the symptoms of acute haemolytic anaemia is presented in Box 2.

BOX 2

CHECKLIST OF SYMPTOMS OF ACUTE HAEMOLYTIC ANAEMIA

- ☐ Back pain
- ☐ Dark (red or black) urine
- ☐ Jaundice
- ☐ Fever
- ☐ Dizziness
- ☐ Breathlessness



Management of side-effects

When a patient presents with signs and symptoms of AHA following the administration of primaquine, the patient should be managed in line with the steps shown in Box 3.

BOX 3

CHECKLIST FOR THE MANAGEMENT OF SIDE-EFFECTS

- ☐ Stop administering primaquine. As primaquine is eliminated rapidly, haemolysis is self-limiting once administration is stopped (2).
- ☐ Give oral hydration.
- ☐ Refer to an inpatient facility.
- ☐ Make a clinical assessment.
- ☐ Check haemoglobin or haematocrit.
- ☐ Check plasma or serum creatinine or urea (blood urea nitrogen) if possible.
- ☐ Give a blood transfusion, if necessary, as follows (3):
 - Haemoglobin < 7 g/dL: transfuse
 - Haemoglobin < 9 g/dL with concurrent haemolysis: transfuse
 - Haemoglobin 7–9 g/dL or > 9 g/dL and no evidence of concurrent haemolysis: careful fluid management with monitoring of urine colour.

Risk–benefit assessment for safe administration of primaquine when G6PD testing is not available

When the G6PD status of a malaria patient is unknown and G6PD testing is not available, a decision to administer primaquine should be based on a risk–benefit assessment at population level (2). Episodes of *P. vivax* malaria, long considered to be relatively benign, can cause significant, lasting morbidity and be life-threatening (10). In an area with a high frequency of relapses, they contribute significantly to transmission, and the the risk–benefit assessment of providing primaquine anti-relapse treatment without testing – even if the G6PD prevalence is relatively high and the capacity of health services to identify and manage primaquine-induced haemolytic reactions is rather poor – may favour starting radical treatment of liver-stage infections at the appropriate dosages (either 14 days or 8 weeks, as applicable).

The individual and public health risk of administering primaquine without G6PD testing may be greater than the benefits in areas with:

- low relapse rates,
- low *P. vivax* incidence rates,
- high G6PD deficiency prevalence rates,

- inconsistent counselling of patients regarding the signs and symptoms of primaquine induced haemolysis and appropriate action
- poor access to health care system and capacity of health facilities to manage acute haemolytic anaemia.

The individual and public health risk of administering primaquine without G6PD testing may be less than the benefits in areas with:

- high relapse rates,
- high *P. vivax* incidence rates,
- low G6PD deficiency prevalence rates,
- consistent and broad coverage of patient counselling regarding the signs and symptoms of primaquine induced haemolysis and appropriate action,
- good access to health care system and good capacity of health facilities to manage acute haemolytic anaemia.

Moreover, countries may consider the following interim guiding principles for progressive deployment of G6PD testing and primaquine for radical cure of all confirmed cases of *P. vivax* and *P. ovale* malaria (15):

- **Countries in the phase of elimination of *P. vivax* malaria or of prevention of re-introduction** (or both) should incorporate G6PD testing into treatment guidelines and ensure that all patients with *P. vivax* malaria who do not know their G6PD status are tested before administration of primaquine anti-relapse therapy. In these phases of a programme, the patient load is extremely low, and patients are treated under close surveillance, often in hospital. Thus, G6PD testing and administration of primaquine for radical cure should be feasible and should be promoted.
- **In countries in which the burden of *P. vivax* malaria and the prevalence of G6PD deficiency are both high,** *P. vivax* patients should continue to be tested for malaria and treated for the blood-stage infection at all levels of the health system, particularly at community level. Administration of primaquine against the liver-stage infection should be guided by the results of G6PD testing. Thus, patients should be referred to health facilities where both G6PD testing and primaquine treatment can be provided and which have the capacity to assess and monitor patients. G6PD testing should be introduced in pilot studies before large-scale deployment. In areas where G6PD testing is not available, generic guidance should be drawn up for primaquine treatment without testing, taking into account the benefits of preventing relapses and the risks associated with giving primaquine (depending on the population prevalence of G6PD deficiency, the severity of the prevalent genotypes and the capacity of and accessibility to health services in which primaquine-induced haemolytic anaemia can be identified and managed); see risk–benefit assessment above.



CONSIDERATIONS IN IMPLEMENTING THE NEW RECOMMENDATIONS

G6PD testing should be incorporated into national treatment guidelines and services made available as the necessary tools become available, possibly with referral of patients from lower to higher levels of health facilities where both G6PD testing and primaquine can be provided (10). A number of qualitative and quantitative G6PD testing methods are available, which are suitable for use in different settings and at different levels of the health care system. Qualitative tests allow classification of an individual as G6PD deficient or G6PD normal, depending on the threshold (usually 30% enzyme activity), while quantitative tests provide the distinct G6PD enzyme activity of each individual.

Quantitative and qualitative G6PD testing methods and preferred product characteristics of point-of-care G6PD tests

Laboratory settings

Two main **quantitative** testing methods are currently used to measure G6PD activity. Both methods, the **spectrophotometric assay** and the **cytochemical assay**, provide precise measurements of G6PD activity, including that of heterozygous women, and allow unambiguous identification of the “intermediate” G6PD status of this population group. These two diagnostic tests are suitable for use in hospitals and laboratories but not for routine use in most field settings, as they require a functioning cold chain, laboratory equipment and skilled workers or are too expensive.

The **fluorescent spot test** has been the recommended **qualitative** screening method for G6PD deficiency in past decades (1). In this test, a blood sample is incubated with glucose-6-phosphate and NADP in the substrate reagent and then spotted onto filter paper. Once dried, the spots on the filter paper are read under long-wave ultraviolet light. The by-product of the chemical reaction, NADPH, fluoresces, and the intensity of the fluorescence is directly proportional to G6PD activity. Hence, this method allows classification of a blood sample from a heterozygous female as “G6PD normal”, “G6PD deficient” or “G6PD intermediate”. The steps in the test and interpretation of its results, as well as the required equipment and costing information, have been summarized (16) with the example of the Trinity Biotech 203-A G6PDH Screening Test Kit. The fluorescent spot test is an affordable G6PD testing method that provides qualitative visual results within minutes. As it requires trained staff and equipment such as long-wave ultraviolet light, a water bath or heat block, a dark box, reagents and controls, it is not suitable as a point-of-care test in the field, and it is most widely used in professional clinical laboratories, blood banks and haematology institutes. The diagnostic performance of a novel qualitative G6PD test should be at least equivalent to that of the fluorescent spot test (1).

Point-of-care G6PD testing

Most malaria patients live and seek care in peripheral health care facilities. Nevertheless, the characteristics of the tests described above limit their routine use in resource-limited, rural, tropical settings. Easy point-of-care tests are required that allow quick diagnostic results in non-laboratory settings. Qualitative lateral flow tests for use at points of care have recently become commercially available;⁶ these require whole blood from a finger-prick and can be performed and interpreted by

6. There are currently no quantitative POC G6PD tests available which have been independently assessed for their performance – however, such products are under development.

health workers at the bedside or in the field in less than 30 minutes. These relatively new products still have some limitations, such as missing control lines, subjective interpretation of test results and incomplete information on thermal stability from studies with small samples sizes. Moreover, most evaluations were made in laboratory and other controlled environments rather than in the field and in the hands of laboratory technicians rather than health workers. Hence, at present, there is only limited evidence for the operational aspects and availability of robust, thermostable, easy-to-use point-of-care G6PD tests for use at lower levels of the health system.

The two point-of-care G6PD tests that were available on the market and had been assessed for the WHO Evidence Review (ERG) Group meeting in October 2014 were the BinaxNOW G6PD test (Alere), approved by the United States Food and Drug Administration, and the CareStart™ G6PD RDT (Access Bio Inc.) (1). Comparative analyses of the diagnostic performance of these tests with that of the fluorescent spot test, which served as the reference standard, are presented in the Annex, in Tables A1–A3; Table A4 lists further characteristics of these two point-of-care G6PD tests.

In the absence of an established quality assurance/quality control mechanism for these novel POC tests, the ERG – in addition to reviewing detection limits – also recommended a set of **preferred product characteristics** (comprising sensitivity, negative predictive value, thermostability, and ease of use) which can be used by countries as guiding principles on minimum desirable product features for procurement (Box 4).

BOX 4

PREFERRED PRODUCT CHARACTERISTICS FOR QUALITATIVE POINT-OF CARE G6PD TESTS

- The **sensitivity** should be > 95% of that of spectrophotometry or equivalent quantitative tests for detecting G6PD enzyme activity at levels < 30% of normal.
- The **negative predictive value** should be > 95%, i.e. provide a 95% probability that the patient has > 30% normal G6PD activity when the diagnostic test indicates that he or she is not deficient.
- The product should be stable at the **temperatures** expected in tropical settings (30–40 °C).
- The test should have a **visual read-out** that clearly distinguishes between “deficient” and “normal” G6PD activity.

In April 2016, the **WHO prequalification** team announced (17) that it was extending its programme to include G6PD deficiency tests. The full prequalification process for a product comprises three components: (i) assessment of the product dossier, (ii) inspection of the manufacturing site, and (iii) evaluation of the product performance (evaluation of a product in a standardized, laboratory-based process, such as the WHO product testing of malaria RDTs). After consultations with experts in the third quarter of 2016 to determine the dossier requirements and evaluation protocols, applications for prequalification of assays intended for G6PD testing will be considered from the fourth quarter of 2016. However, the first WHO-prequalified products are not expected on the market before the end of 2017 and beyond. In the meantime, manufacturers of relevant in vitro diagnostics may submit an expression of interest to the **Expert Review Panel for Diagnostics** (ERPd) established by the



Global Fund to Fight AIDS, Tuberculosis and Malaria and UNITAID and coordinated by WHO. Most recently, the CareStart™ G6PD RDT has been reviewed by the ERPD and approved as a Category 3 product, i.e. it is only recommended for procurement with Global Fund money on a conditional and time-limited basis, which requires approval on a case by case basis; countries need to include robust safeguards in their purchase requests supporting the safe utilization of the product – detailed requirements are currently under development.

Introducing / expanding a G6PD testing system at country level

National malaria control programmes should progressively introduce or extend G6PD testing to areas in which it is currently not done, taking into consideration lessons learnt from small-scale projects. The introduction of point-of-care G6PD tests should be accompanied by quality assurance, training, supervision and behaviour change communication as well as monitoring of the feasibility, acceptability and ease of use of the tests. The checklist in Table 3 provides further guidance on aspects to be considered in countries introducing these tests.

TABLE 3
Checklist for the implementation of a G6PD testing system at country level

REQUIREMENTS	DESCRIPTION
Policy framework	
National treatment guidelines	<input type="checkbox"/> G6PD testing should be included in the national treatment guidelines (10).
Levels of G6PD testing and primaquine administration; referral system	<input type="checkbox"/> The level of the health care system at which G6PD testing and primaquine administration is effected should be determined, considering health facilities, where patients can be assessed and monitored for possible drug-induced haemolytic anaemia. If required, a referral system should be in place for G6PD testing and radical cure with primaquine after <i>P. vivax</i> infection has been diagnosed at a peripheral health facility and treatment started with medicines against the blood stage infection. Referral may, as a bare minimum, also require a fluorescent spot test in a laboratory in which the necessary skills and equipment are available.
Health care system	
Develop plan for pilot implementation and progressive scale-up of G6PD testing and primaquine administration	<input type="checkbox"/> In close collaboration with a national technical working group, develop a plan for pilot implementation and progressive scale-up of G6PD testing, in line with the national treatment guidelines. Prepare and disseminate clear guidance on primaquine administration without G6PD testing for use in all areas at which point-of-care G6PD testing will initially not be available.
Health care personnel	<input type="checkbox"/> Prepare training materials, including on the testing and treatment algorithm, job aids for the performance and interpretation of G6PD tests, a checklist for patient counselling, job aids for the identification of acute haemolytic anaemia, and supervision checklists. Translation into local languages should be considered, particularly for material intended for patients. <input type="checkbox"/> Initial training and regular supervision of health care workers in correct preparation and reading of point-of-care G6PD tests is essential.

REQUIREMENTS	DESCRIPTION
Recording of G6PD status	<input type="checkbox"/> Prepare or adapt reporting material to create permanent records of the G6PD status of patients. (Some countries, e.g. Malaysia and the Philippines, screen newborns for G6PD deficiency and record the results on personal medical cards.)
Management of side effects	<input type="checkbox"/> Strengthen the capacity of health services to properly manage side-effects of primaquine, particularly the management of acute haemolytic events (including blood transfusion services).
Behaviour change communication	<input type="checkbox"/> Provide information, education and communication, and create demand among health care providers and patients.
Pharmacovigilance	<input type="checkbox"/> As primaquine becomes more widely used, it may be combined more frequently with other medicines that can also induce haemolytic anaemia in G6PD-deficient patients. Therefore, pharmacovigilance should be strengthened in areas where primaquine will be used. This will allow to improve knowledge of drug safety, increase the ability to detect rare side-effects and improve the confidence of health care professionals and consumers.

Quality assurance and quality control of commodities

POC G6PD tests	<input type="checkbox"/> Point-of-care G6PD tests should be selected according to the preferred product characteristics (see Box 4). <input type="checkbox"/> Temperature should be monitored during shipment, storage and distribution and kept within the manufacturer's specifications in order to avoid undue exposure to high temperatures, which may affect the performance of the tests. Guidance documents for rapid diagnostic tests for malaria (18, 19) could be used as a reference. <input type="checkbox"/> In April 2016, WHO announced that it would extend its prequalification programme to review G6PD tests. In the meantime, an expert review panel for diagnostics has been established by the Global Fund to Fight AIDS, Tuberculosis and Malaria and UNITAID, coordinated by WHO (see above page 16).
Primaquine	<input type="checkbox"/> Anti-relapse medicine should be procured in accordance with internationally agreed quality standards (see above page 10). <input type="checkbox"/> Pre- and post-shipment quality control can be undertaken at WHO prequalified laboratories.*

Resource implications

	<input type="checkbox"/> Consider the time requirements for planning, procuring, preparing training, supervision and data management tools, strengthening the referral system and transfusion services. <input type="checkbox"/> Plan pilot projects to determine the requirements for training and supervising staff. <input type="checkbox"/> Consider all costs, such as for products, shipping, storage, distribution, training and supervision and quality control, including health systems costs for management of patients with acute haemolytic anaemia. <input type="checkbox"/> Identify a funding source for progressive extension of G6PD testing throughout the country.
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* The WHO List of Prequalified Quality Control Laboratories, which in its March 2016 version contains 41 laboratories in the six WHO regions, is available at the following link: http://apps.who.int/prequal/lists/PQ_QCLabsList.pdf.



FURTHER RESEARCH

Further research is required to answer the following (non-exhaustive) list of questions:

- How acceptable are point-of-care G6PD tests to health workers for deciding to give primaquine?
- What are the most effective and cost-effective operational strategies for linking a diagnosis of malaria, G6PD testing and radical cure of *P. vivax* or *P. ovale* infections, including a functioning referral system?
- How accurate are G6PD tests when conducted routinely by health workers?
- What is the optimal training (e.g. materials, duration) for health workers to ensure that they perform G6PD testing accurately and safely?
- What are best options for long-term recording of a person's G6PD status?
- What are the most effective approaches for monitoring adverse events?
- How do geography and genetic diversity affect test performance?

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ANNEX. CHARACTERISTICS OF QUALITATIVE G6PD TESTS

Table A1 shows a comparison of the diagnostic performance of qualitative tests for G6PD deficiency, from published studies.

TABLE A1
Diagnostic performance of qualitative tests for G6PD deficiency

REF	TEST	SPECIMEN	GOLD STANDARD	THRESHOLD	NO. OF SAMPLES	NO. DEFICIENT	HETERO-ZYGOTES	SENSITIVITY (%)
1	WST8/1-methoxy PMS	Finger-prick/DBS	R and D diagnostics	< 60% median of males and females	235	30 (all > 10% normal)		72
2	FST	Venous	Trinity	Median of normal males	214	23	25	100 (30%) 91 (60%) 100 (10%)
3	FST	Venous	BIOLABO SA/	10% genotype	295	42	34	43 (Genotype)
4	FST	?	Genotype	All normal by FST	461	27	61	All misclassified by FST
5	Binax NOW	Venous	Trinity	4.0 U/g Hb	246	50	–	98
6	Binax NOW	Venous	Trinity	< 60% median of males and females	356	11	–	54.5
2	Binax NOW	Venous	Trinity	Median of normal males	214	23	25	100 (30%) 83 (60%)
7	first-generation Care Start®	Venous	Trinity	Lower limit from 174 normal subject (~30%)	903	97	–	68
8	Care Start	Venous	Trinity	Mean from > 4.56 IU/g Hb and [Hb] > 12 g/DI	456	46 (< 30%)	–	90 (< 10%) 84.8 (< 30%)

PMS, phenazinium methylsulfate; FST, fluorescent spot test; DBS, dried blood spot

Over the past few decades, the fluorescent spot test has been the recommended qualitative test for screening for G6PD deficiency (9). The Expert Working Group agreed that the diagnostic performance of novel qualitative G6PD tests should be at least equivalent to that of the fluorescent spot test.

Because of the limitations of the fluorescent spot test and the temperature restrictions for use of the Binax NOW G6PD test (Alere), one of two commercially available point-of-care G6PD tests, the Group reviewed independent evaluations (published and unpublished) of the only other commercially available test potentially appropriate for use in tropical settings in which *P. vivax* is endemic, namely the CareStart® G6PD test (Access Bio Inc.). Table A2 lists screening tests for males, and Table A3 lists those for females.

TABLE A2

Assessment of different commercially available G6PD diagnostic screening tests in male subjects from different countries

STUDY/PI	TEST	SAMPLE TYPE	SETTING	OPERATOR	READER ASSESSMENT	TEMP (°C)	SENSITIVITY (%) / CI	SPECIFICITY (%) / CI	PPV (%)	NPV (%)	PREVALENCE (%) / SAMPLE SIZE	REFERENCE STANDARD
Cambodia/ D. Menard^{*1}	CareStart® v2	Venous & Capillary	Mobile lab	Technician	2 independent readers, if discordant, a third reader	26–29	100.0	98.7	92.2	100.0	15.0/392	G6PD Quantitative Trinity Biotech
	CareStart® v2	Venous	Lab	Technician	2 independent readers, if discordant, a third reader	28–29	87.5	100.0	100.0	89.7	9–18/150	G6PD Quantitative Trinity Biotech
Thailand/ G. Bancone^{*2}	R&D Diagnostic	Venous	Lab	Technician	2 independent readers, if discordant, a third reader	28–29	100.0	100.0	100.0	100.0	9–18/150	G6PD Quantitative Trinity Biotech
	R&D Diagnostic	Capillary	Lab	Technician	2 independent readers, if discordant, a third reader	28–29	96.0	100.0	100.0	96.3	9–18/150	G6PD Quantitative Trinity Biotech
Indonesia/A. Satyagraha^{*3}	CareStart® v2	Venous	Field	Technician	1 reader, if unsure, another reader	29–34	100.0/ (100.0–100.0)	98.7/ (97.3–100.0)	89.0/ (77.0–100.0)	100.0/ (100.0–100.0)	9.2/260	G6PD Quantitative Trinity Biotech
	FST Trinity Biotech	Venous in EDTA	Lab	Technician	2 readers, if discordant, a third reader	26–29	91.7 / (80.6–100.0)	92.4/ (89.0–95.8)	55.0/ (40.0–70.0)	100.0/ (100.0–100.0)	8.5/260	G6PD Quantitative Trinity Biotech
Brazil/M. VG Lacerda^{*4}	CareStart®	Venous in EDTA	Lab	Technician	2 readers, if discordant, a third reader	19–26	61.5	98.3	42.1	99.2	1.9/674	G6PD Quantitative Pointe Scientific

PI, principal investigator; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value

* Based on the result of 30% cut-off value of normal G6PD activities

1 Based on Roca-Feltre et al. (10)

2 Based on Bancone et al. (11)

3 The G6PD prevalence was based on the FST or RDT results against the reference standard result.

4 Study in males only.



TABLE A3.
Assessment of different commercially available G6PD diagnostic screening tests in female subjects from different countries

STUDY/PI	TEST	SAMPLE TYPE	SETTING	OPERATOR	READER ASSESSMENT	TEMP (°C)	SENSITIVITY (%)	SPECIFICITY (%)	PPV (%)	NPV (%)	PREVALENCE/ SAMPLE SIZE	REFERENCE STANDARD
Cambodia/ D. Menard ^{*1}	CareStart® v2	Venous & Capillary	Mobile lab	Technician	2 independent readers, if discordant, a third reader	26–29	100.0	94.5	36.6	100.0	3.6/419	G6PD Quantitative Trinity Biotech
		Venous										
Thailand/ G. Banccone	CareStart® v2	Capillary	Lab	Technician	2 independent readers, if discordant, a third reader	28–29	100.0	82.7	60.6	100.0	–	G6PD Quantitative Trinity Biotech
		Venous										
Thailand/ G. Banccone	R&D Diagnostic	Capillary	Lab	Technician		28–29	100.0	97.4	91.7	100.0	–	
		Venous										
Indonesia/A. Satyagraha ^{*2}	CareStart® v2	Venous	Field	Technician	1 reader, if unsure, another reader	29–34	83.3/ (53.5–100.0)	92.7/ (90.0–95.5)	17.0/ (3.0–30.0)	100.0/ (99.0–100.0)	1.4/350	G6PD Quantitative Trinity Biotech
		Venous										
Indonesia/A. Satyagraha ^{*2}	FST Trinity Biotech	Venous in EDTA	Lab	Technician	2 readers, if discordant, a third reader	26–29	100.0/ (100.0–100.0)	92.2/ (89.3–95.0)	18.0/ (5.0–31.0)	100.0/ (100.0–100.0)	1.7/350	G6PD Quantitative Trinity Biotech
		Venous										

PI, principal investigator; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value

* Based on the result of 30% cut-off value of normal G6PD activities.

1 Based from Roca-Fellner et al. (10)

2 The G6PD prevalence was based on the FST or RDT results against the reference standard result.

Table A4 summarizes the relevant characteristics of the two commercially available point-of-care G6PD lateral flow tests for their use under field conditions.

TABLE A4
Characteristics of commercially available qualitative point-of-care G6PD tests

	CARESTART™ G6PD	BINAXNOW G6PD
Thermostability		
• Storage temperature	• Room temperature (no cold chain required)	• 2°C – 30°C (no cold chain required)
• Assay temperature	• 18°C ~ 32°C	• 18°C – 25°C
Test result		
• Interpretation	• Normal versus deficient	• Normal versus deficient
• Time	• 10 minutes	• < 10 minutes
Laboratory skills required	No	No
Laboratory equipment required	No	No
Low cost of test	Yes	No

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**Testing for G6PD deficiency for safe use of
primaquine in radical cure of *P. vivax* and *P. ovale*
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