

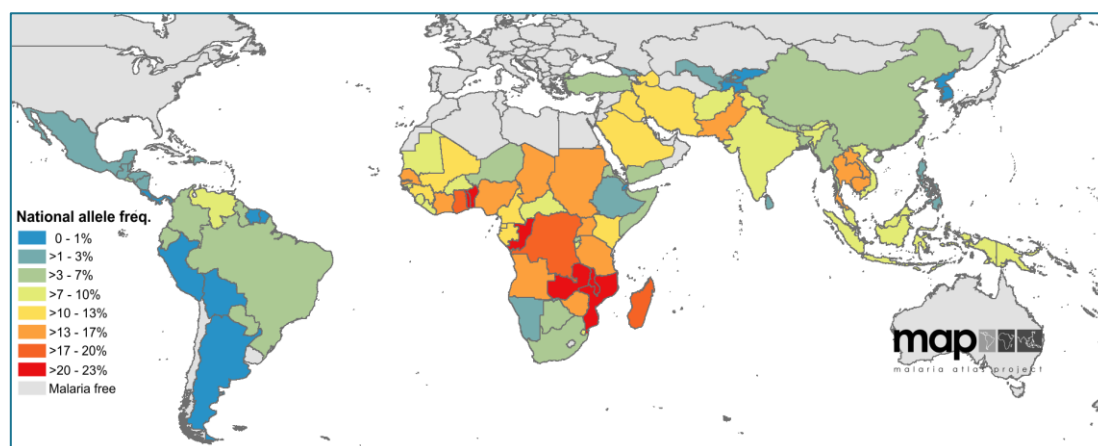
Point-of-care G6PD testing to support safe use of primaquine for the treatment of vivax malaria

WHO Evidence Review Group meeting report
8–9 October 2014, WHO/UNAIDS Building, Geneva, Switzerland

Background

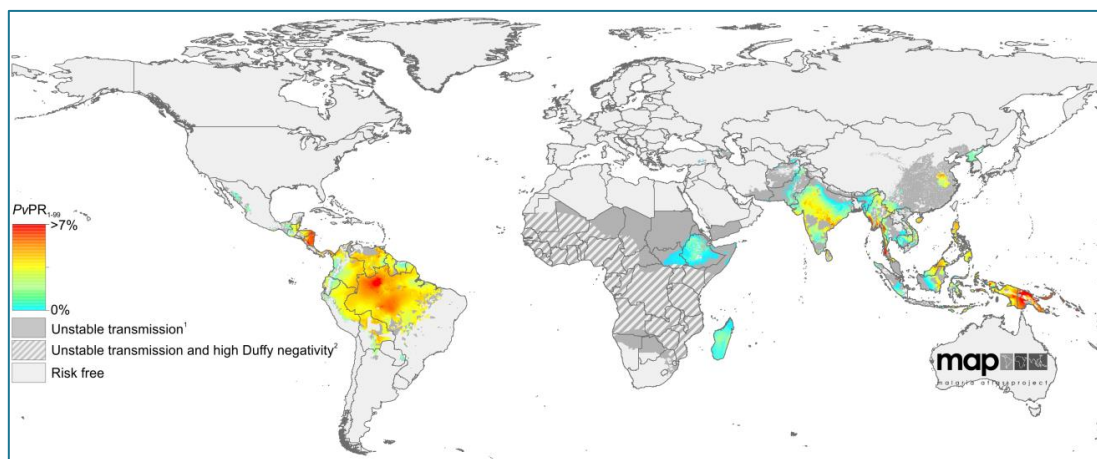
The problem

The drug primaquine has been in continuous use since 1952 for the prevention of relapse of *Plasmodium vivax* and *P. ovale*. Primaquine has haemolytic toxicity in those who are deficient in glucose-6-phosphate dehydrogenase (G6PD); also, where most malaria patients live, it is often not practical to test for G6PD deficiency and supervise a 1–2 week course of therapy. Nevertheless, there are no alternative therapeutic options to prevent *P. vivax* relapse (1). A daily dose of 0.25 or 0.5 mg/kg for 14 days induces acute haemolytic anaemia (AHA) in patients with inborn deficiency of G6PD (2). This genetically heterogeneous X-linked recessive condition affects over 400 million people globally (see Maps 1 and 2 below).



Map 1 National prevalence of G6PD deficiency in males

Across malaria endemic countries, the mean allele frequency of G6PD deficiency is estimated to be 8.0% (interquartile range [IQR]: 7.4–8.8). Since the G6PD gene maps to the X chromosome, the frequency of G6PD deficiency in males is the same as the allele frequency. The frequency of G6PD-deficient females was estimated as the sum of all female homozygotes plus the subset of heterozygotes who have 30% or less of normal G6PD activity (see Table 1). In total, this amounts to about 350 million individuals with G6PD deficiency. Figure reproduced from Howes RE et al. (3).



Map 2 Spatial distribution of *Plasmodium vivax* malaria endemicity in 2010

The limits of transmission (risk free or unstable annual parasite index [API] <0.1 per 1000 per year/stable transmission) are defined by API data, supplemented by further data on temperature and aridity masks. Parasite rate population surveys are used in a Bayesian geostatistical framework to model the annual mean endemicity of *P. vivax* across areas of stable transmission, represented in colour and standardized to all ages (1–99 years). Figure reproduced from Gething PW et al. (4).

Since 1956, G6PD deficiency has been recognized as the cause of sensitivity to primaquine (5). Whenever it is not possible to test for G6PD deficiency before administering primaquine, balanced decision-making is required, weighing the potential risk of harm (i.e. of AHA) upon primaquine intake to a patient who may be G6PD deficient against the risk of harm due to multiple clinical relapses of acute *P. vivax* malaria when primaquine is not administered. Additionally, the contribution of repeated relapses of *P. vivax* infection on morbidity, mortality and transmission needs to be considered (6, 7).

Thus, knowing whether a patient is G6PD deficient or not is key to realizing the potential of primaquine anti-relapse therapy to improve *P. vivax* case management and contribute to interruption of transmission. In practical terms, this requires either that the result of a G6PD test done at birth or otherwise is readily available, or that point-of-care (POC) G6PD testing is done following a diagnosis of vivax malaria (7). To address this, the WHO Global Malaria Programme, convened an Evidence Review Group (ERG) to examine evidence related to new technologies and devices for testing G6PD deficiency at the POC.

Objectives of the ERG

The specific objectives of this ERG were to apply available evidence in order to answer the following questions:

1. What is the lowest level of prevalence of G6PD deficiency below which there is no need to test for G6PD deficiency before giving primaquine for radical treatment of vivax malaria?
2. What test(s) should be used for classifying males as G6PD normal or deficient?
3. What test(s) should be used in order to identify presumably heterozygous females who are at clinical risk of PQ-induced haemolysis. This requires knowledge on the level of G6PD deficiency (proportion of G6PD deficient red cells, which can vary in heterozygous females between 0% and 100%) at which primaquine will induce a clinically significant haemolysis when given at the standard doses recommended for vivax radical cure.

4. When a male is classified as G6PD deficient, or when a female is classified as a heterozygote with a clinically-relevant proportion of G6PD deficient red cells, or when G6PD heterozygosity cannot be safely diagnosed from female cases, what is the appropriate management of vivax malaria?
 - a. No primaquine.
 - b. Primaquine at a lower dose but for an extended period.
 - c. Primaquine at the standard dose but under medical supervision.

Evidence reviewed

In preparation for the meeting, WHO commissioned a review of the literature on primaquine-related haemolysis risk in G6PD-deficient heterozygous females, kindly completed by Dr J. Recht. In this review, PubMed was searched using the terms “primaquine”, “G6PD”, “G6PD activity”, “G6PD deficiency”, “hemolysis” and “heterozygote/ heterozygous”. Relevant articles also included those reviewed for a WHO ERG meeting held in 2012 on the safety of gametocytocidal use of primaquine in *P. falciparum* infection (8). In addition to published literature identified via PubMed and EmBase searches, studies in press and unpublished evidences were made accessible to the members of the ERG. For G6PD tests, recently published reviews were considered (9, 10), providing comparative analysis of the performance of available tests. The evidence reviewed focused on available POC rapid diagnostic tests for G6PD, appropriate for use in tropical and resource-limited settings. A specific qualitative G6PD test (CareStart™) was considered in view of its potential application in resource-limited tropical settings where *P. vivax* is endemic. Also, efforts were made to include unpublished but completed studies evaluating this product, according to a common template that would allow comparable assessment of test performance.

The rapporteurs prepared the present document from several of the pre-reads shared with the participants before the ERG meeting, and from discussions during the meeting. The report was then reviewed in detail by all participants, and any inputs provided were taken into consideration in finalizing the review.

The deliberations of the evidence review group were guided by the knowledge of primaquine-induced toxicity in G6PD-deficient patients, the limitations of historical experience and of published data on safety of primaquine anti-relapse therapy for vivax patients with G6PD deficiency, and the results of recent studies evaluating the performance of qualitative POC G6PD.

Report of the evidence review group

Burden of relapses due to *P. vivax* and health risks

The individual and public health threats posed by relapses due to untreated *P. vivax* liver stage infection need to be taken into account when discussing the risks and benefits of primaquine therapy. The timing and risk of relapse varies widely across geographic regions (see Figure 1 below).

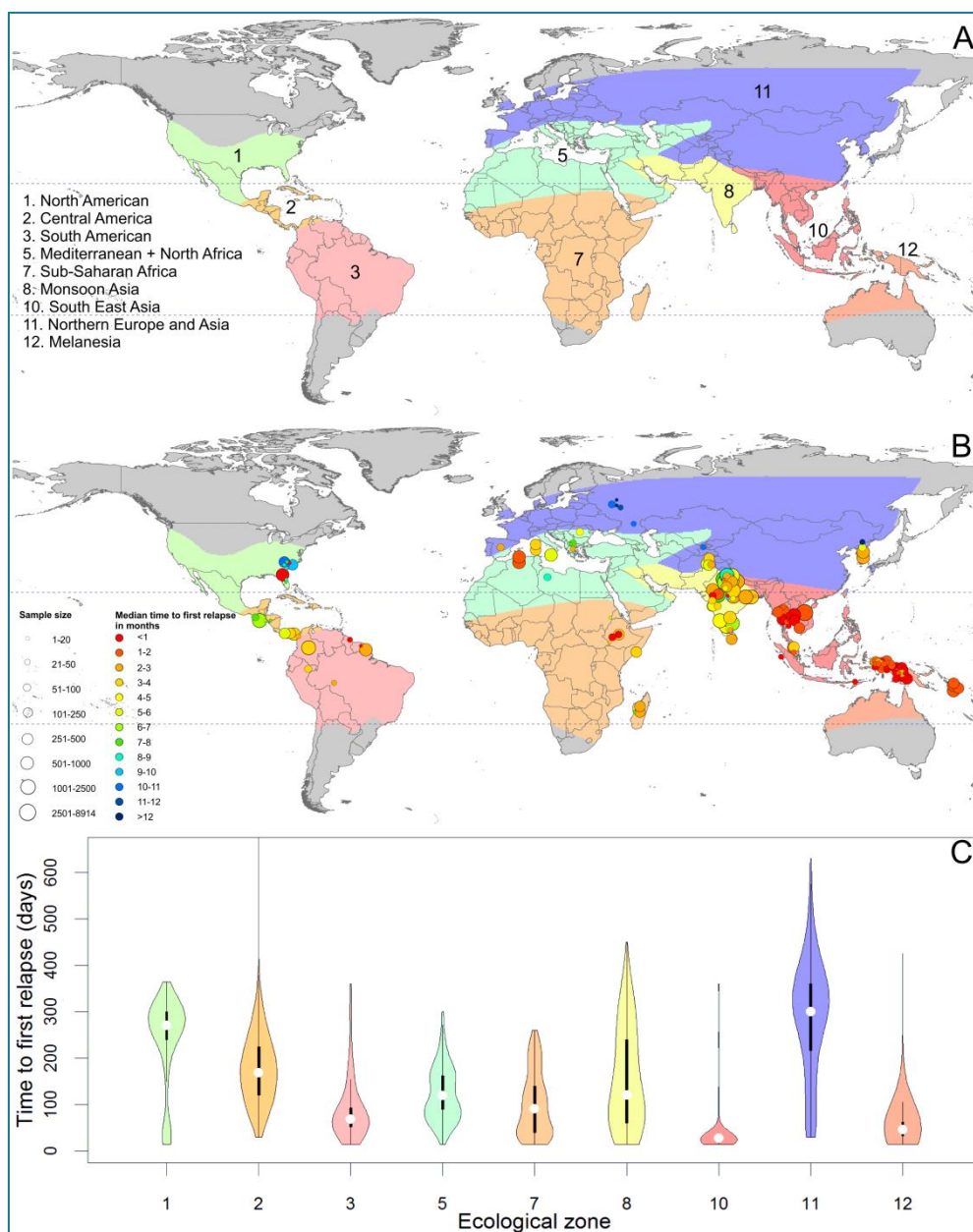


Figure 1 Zones of malaria, surveys used and time to relapse

Map showing ecological zones of malaria (top) and surveys used (middle) to model the time to first relapse in individuals from each zone, illustrated in the graph by violin plots (bottom). From Battle K, et al. (11).

Figure 1 above shows that the pattern of rapidly relapsing strains in tropical areas of Latin America and Africa is similar to the relapse behaviour represented by Chesson-like strains of *P. vivax* of tropical Southeast Asia and Oceania. The variation in risk and timing of relapse is key to weighing the specific local risks and benefits of primaquine therapy.

A recent clinical trial of primaquine therapy in Indonesian soldiers provides a clear picture of the burden imposed by vivax malaria not treated with primaquine (12). In this study, nearly 80% of the male subjects who did not receive primaquine anti-relapse therapy relapsed within 6 weeks. This represents an incidence of five relapses/person-year, roughly equivalent to the attack rates of *P. falciparum* in high transmission areas of sub-Saharan Africa. This and other studies (13)

illustrate the substantial burden of recurrent illness and, by implication, the contribution to vivax transmission by the hypnozoite reservoir, particularly of tropical strains.

Episodes of *P. vivax* malaria, long considered relatively benign, can cause significant and lasting morbidity and even mortality. Recent studies have challenged the dogma that acute vivax malaria is rarely life-threatening (14–18). This potential harm must also be considered in the risk–benefit assessment of primaquine therapy. Multiple relapses may contribute substantially to the mortality risks relative to falciparum malaria. In Figure 2, the odds ratio of a fatal outcome among patients hospitalized with a primary diagnosis of vivax malaria relative to falciparum malaria often included 1.0, and the average among them was 0.64 (0.52–0.78).

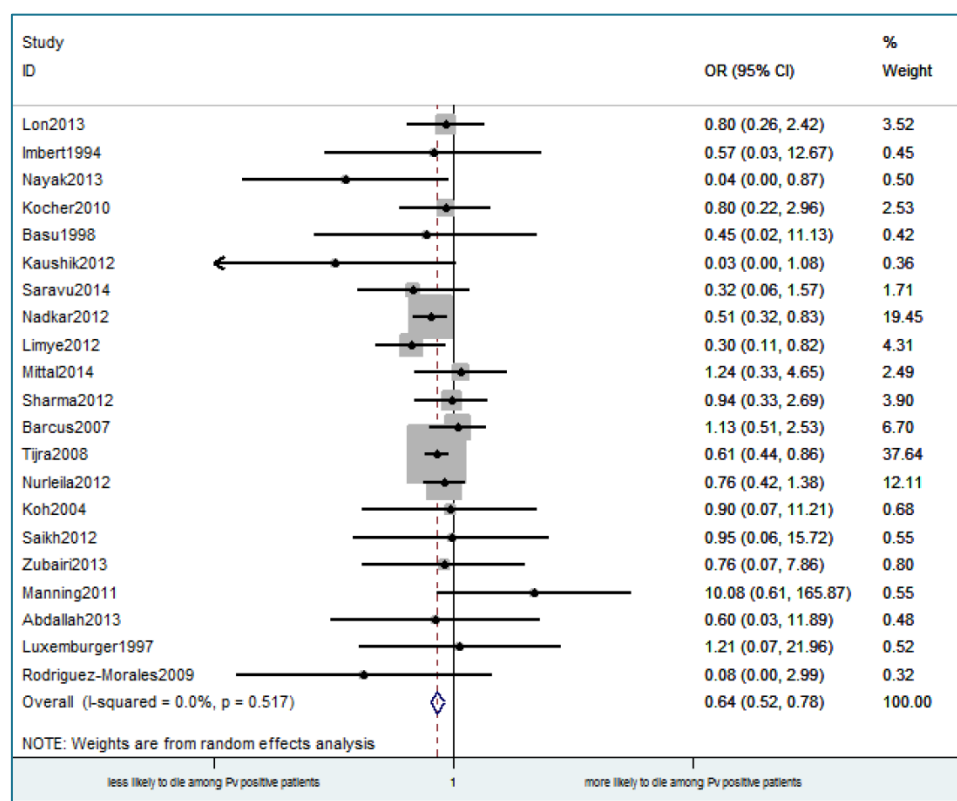


Figure 2 Odds ratio of death as an outcome – comparison of vivax and falciparum malaria

Chart plots the odds ratio (x-axis) of death as an outcome in patients admitted to hospital with a primary diagnosis of vivax malaria relative to the same in patients with a diagnosis of falciparum malaria. An odds ratio of 1.0 represents no difference. Figure reproduced from the WHO Technical briefing on control and elimination of *Plasmodium vivax* malaria (19).

These findings serve to underline an important consideration; namely, that although the administration of primaquine in the absence of knowledge of G6PD status can pose serious hazard, so too does withholding primaquine therapy. In settings of high risk of relapse of relatively virulent strains and poor access to good health care, either decision may result in significant consequences. This is the primaquine–G6PD dilemma in the treatment of vivax malaria.

Country policies on primaquine anti-relapse therapy and G6PD testing

Most countries with endemic *P. vivax* recommend anti-relapse therapy with primaquine, in line with WHO recommendations, although four countries (Algeria, Cambodia, Ethiopia and Somalia) do not recommend its use (Figure 4). Most of those countries that do recommend primaquine

recommend the standard 0.25 mg/kg daily dose for 14 days, though some recommend 0.5 mg/kg daily for either 7 or 14 days. A recent meta-analysis suggested efficacy of the 0.25 mg/kg daily dosing regimen (14 days) for temperate strains, and of the 0.5 mg/kg daily dosing regimen (14 days) for tropical frequent relapsing *P. vivax* (20). Nonetheless, the programmes of most vivax endemic countries adhere to the lower dose (0.25 mg/kg/day for 14 days) on the basis of the higher risk of more serious harm at the more efficacious higher total dose. Only Iran has adopted the weekly primaquine regimen of 0.75 mg/kg once weekly for 8 weeks without G6PD testing, as shown in Figure 3.

The application of these recommendations in patients, most with unknown G6PD status, varies among nations, and even within nations among health-care providers. Data on antimalarial tablets suggests that, in some countries where primaquine is recommended without G6PD testing, prescription rates are as low as 10% of vivax cases treated with an antimalarial drug.

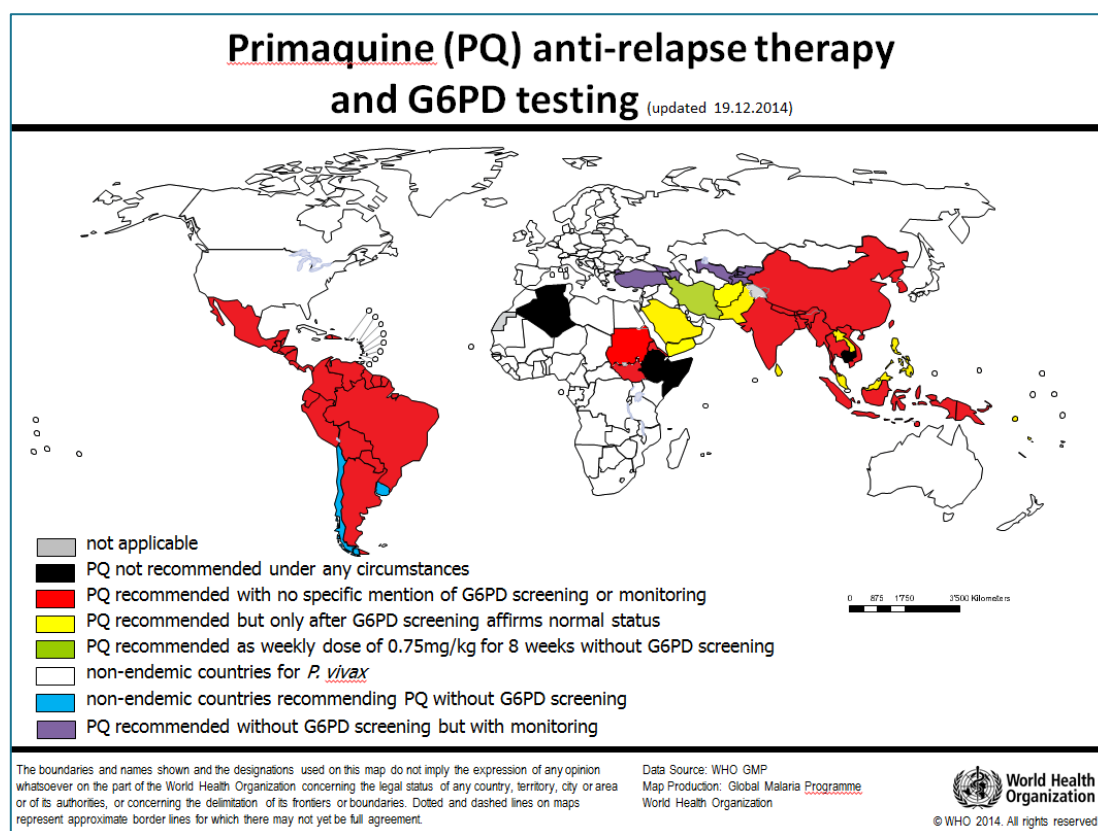


Figure 3 Primaquine therapy as reflected in national treatment guidelines globally

WHO/GMP Survey, 2014 – last updated 19.12.2014.

The reasons for not prescribing primaquine anti-relapse therapy are multifold and complex, but risk of haemolytic toxicity among G6PD-deficient patients is probably the dominant factor. The lack of field-adapted tests, the limited availability of primaquine in many settings and the difficulties in adhering to a 14-day regimen all contribute to what is probably low primaquine effectiveness in many settings.

The problem of female heterozygotes

The ERG considered the implications of G6PD deficiency being an X-linked disorder. This creates two distinct genotypes in males: hemizygous G6PD normal (wild type), and hemizygous G6PD

deficient; and three distinct genotypes in females: homozygous G6PD normal (wild type), and heterozygous and homozygous G6PD deficient (see Table 1). Thus, there are five genotypes in total (two in males and three in females), but only three phenotypes: normal, deficient and intermediate (i.e. from normal to deficient in heterozygous females).

Table 1 Relationship between G6PD genotypes, enzyme activity in red cells, recommended nomenclature and clinical implications with respect to primaquine

Genotype	Sex	G6PD activity	Phenotypic nomenclature	Primaquine sensitivity
XY – wild type	Male	Normal	Normal	No
XX – wild type	Female	Normal	Normal	No
X*Y – hemizygote	Male	<30% of normal	Deficient	Yes
X*X* – homozygote	Female	<30% of normal	Deficient	Yes
X*X – heterozygote	Female	<30% of normal	Deficient	Yes
X*X – heterozygote	Female	Between 30% and 80% of normal	Intermediate	Possible
X*X – heterozygote	Female	>80% of normal	Normal	Unlikely

NB – The classification of “intermediate” is possible with quantitative testing but does not yet inform the decision of whether to proceed with primaquine therapy or withhold it.

X chromosome inactivation (or “lyonization”, from the name of Mary Lyon who discovered this phenomenon) has major implications in relation to G6PD testing and primaquine toxicity. During embryonic development, one of the two X chromosomes becomes inactivated in every somatic cell in an apparently random manner. However, once inactivation has taken place, the active or inactive state of the X chromosome is faithfully maintained in the progeny of each cell. If a female is heterozygous for a normal and a G6PD deficiency allele, this results in mosaicism: that is, the coexistence of a population of red blood cells (RBCs) that have normal G6PD activity and a population that are G6PD deficient. The relative proportion of deficient to normal RBCs ranges from 0% to 100% in individual heterozygous females, according to a normal (Gaussian) distribution (see Figure 4 below).

This is highly relevant to G6PD testing for the purpose of primaquine therapy. There are two issues:

- *Biological* – In heterozygous females, a cytochemical (flow cytometry or microscopy based) assay of G6PD activity can determine what proportion of RBCs are G6PD deficient; correspondingly, a quantitative assay result will reflect this. At the lower end of the spectrum there will be overlap between heterozygotes and homozygous deficient females; at the upper end of the spectrum there will be overlap between heterozygotes and homozygous normal females (see Figure 5 below).
- *Technical* – Most heterozygotes will have a G6PD activity between 30% and 80% of normal, and in this range the performance of all qualitative screening methods and devices may be ambiguous. In other words, RDTs are mostly meant to give a YES or NO result; however, in heterozygotes, the result may be ambiguous because the enzyme activity is intermediate (see Table 1 above).

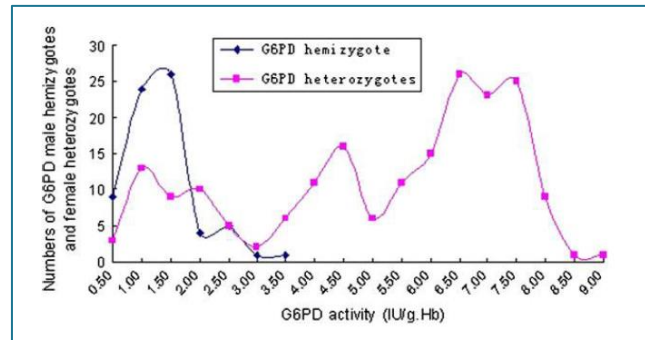


Figure 4 Distribution of male hemizygotes and female heterozygotes for G6PD deficiency along the x-axis of G6PD enzyme activity levels

The males are relatively narrowly distributed at the low end of activity, whereas female heterozygotes appear at all levels of activity. From Jiang WY, et al. (21).

In some cases, a rapid diagnostic test (RDT) may classify as normal a heterozygous female who has a substantial proportion of RBCs vulnerable to primaquine-induced haemolysis. This is a conspicuous limitation of RDT (qualitative) G6PD tests, and has been carefully considered in ERG deliberations.

Classification of G6PD deficiency

Variants and degrees of severity

In G6PD-normal patients, primaquine is a remarkably safe and well-tolerated drug with high efficacy in preventing relapses of *P. vivax*. A recent trial of the 0.5 mg/kg primaquine daily dose for 14 days given after therapy with dihydroartemisinin-piperaquine for acute vivax malaria showed good safety and tolerability, with 98% efficacy against relapse (12).

Daily primaquine therapy induces AHA in G6PD-deficient patients. The severity of the AHA is variable in relationship to the enzyme half-life and consequent residual level of enzyme activity associated with individual genetic variants of G6PD. Historically, the severity of G6PD deficiency has been classified as mild or severe, largely depending on the reduction in activity of the G6PD enzyme associated with each variant. In the 1980s, WHO adopted a classification scheme that identified common G6PD deficient variants as either class II (activity <10% of normal) or class III (activity >10% of normal) (22). The prototype of class II was G6PD Mediterranean and the prototype of class III was G6PD A- (the common African variant). Classification of other variants as class II or class III has been often based on few samples tested in different laboratories. The relationship between enzyme activity and sensitivity to AHA is based on a relatively small number of studies of healthy adult subjects challenged with primaquine.

The physiological difference between African A- and Mediterranean variants may be appreciated from Figure 5.

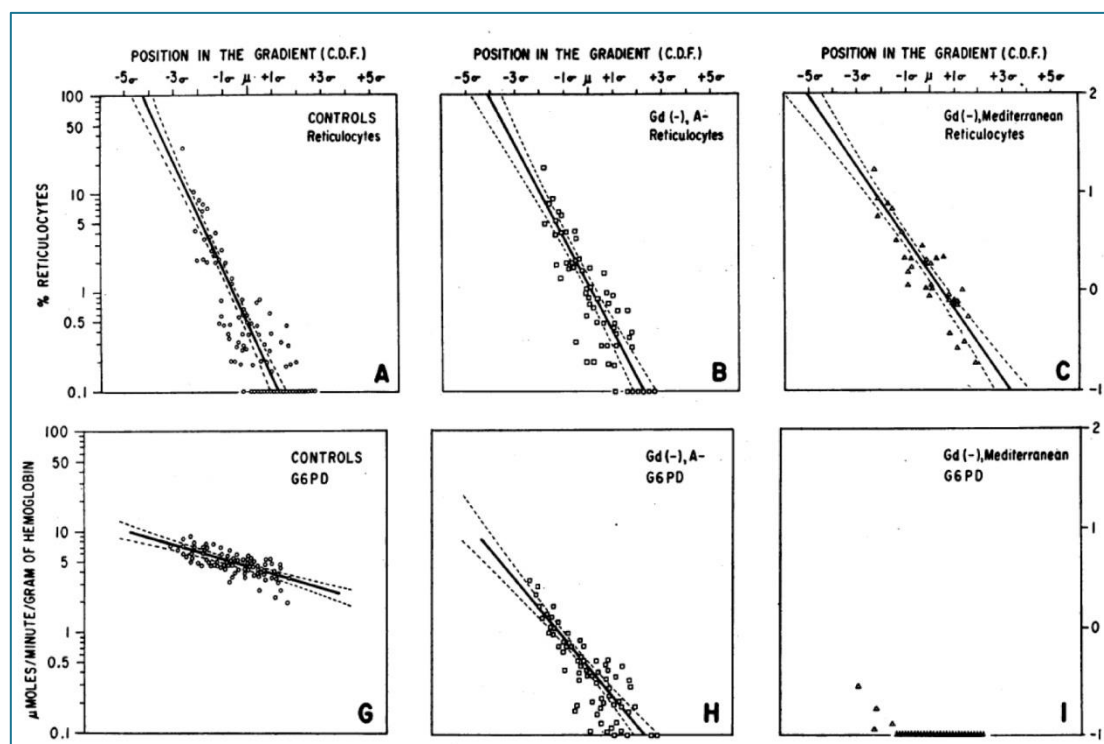


Figure 5 G6PD activity in relation to age of red blood cells

The y-axis (on a log scale) shows G6PD enzymatic activity and the x-axis (linear) the age of RBCs. Upper panels illustrate the proportion of reticulocytes as RBCs age in G6PD normal (panel A), African A- G6PD-deficient (panel B), and Mediterranean G6PD-deficient (panel C) subjects. Lower panels illustrate the decay of G6PD activity as RBCs age in G6PD-normal (panel G), African A- G6PD-deficient (panel H), and Mediterranean G6PD-deficient (panel I) subjects. Taken from Piomelli et al (23).

The findings illustrated in Figure 6 concur with the few clinical studies of primaquine challenge in subjects having A- or Mediterranean variants. Those with the A- variant suffer haemolysis of approximately 30% of RBCs within a week of daily doses of 0.5 mg/kg, but thereafter recover to baseline haematocrit, and sustain this level despite ongoing dosing for many weeks (24). This compensation is explained by the contingency whereby the oldest RBCs are destroyed and then are replaced by primaquine-tolerant younger RBC populations, which exhibit tolerance even to continued high-dose (30 mg daily) primaquine exposure. In contrast, subjects with the Mediterranean variant do not appear to develop tolerance, because even much younger RBCs (see Figure 6, panel I) have insufficient residual G6PD activity to tolerate the oxidative stress of primaquine. As a result, the haemolytic anaemia is more severe and not self-limiting.

Thus, although there is diversity of G6PD deficiency levels, the risk of AHA upon primaquine therapy applies to all G6PD-deficient variants. The assumption that daily primaquine therapy is relatively safe in patients with the African A- variant is incorrect, because the broadly used characterization of A- variant as “mild and self-limiting” is derived from only a few studies undertaken in small numbers of otherwise healthy male volunteers (25). There are three types of G6PD A- in Africa, and it is unknown what the genotype was in subjects included in the primaquine challenge studies undertaken in 1950s and 1960s (although by far the most frequent genotype is that with the G202A nucleotide replacement (26)). Indeed, two fatalities in Brazil attributed to primaquine-induced haemolysis, both confirmed at autopsy, carried the A- variant (M. Lacerda, personal communication).

Thus, the classification of variants of G6PD deficiency as “mild” and “severe” for the purpose of guiding clinical decisions should be abandoned. Daily primaquine hyponozoitocidal therapy

(0.5 mg/kg daily for 14 days) induces potentially life-threatening AHA in patients with all of the known G6PD variants, including the A- variant. Further, in view of the limitations of pharmacovigilance in most endemic countries, the relatively small numbers of published reports of serious harm caused by primaquine therapy prescribed without G6PD testing cannot be accepted as evidence of absence of risk of potential harm. In many endemic settings of limited clinical capacities, it seems likely that primaquine-induced AHA may often be inappropriately attributed to the malaria attack or otherwise not understood as being linked to primaquine therapy.

Levels of G6PD activity

The following definitions were adopted in the deliberations of the ERG.

- **In males:** Any male who has red cell G6PD activity less than 30% of the normal mean must be regarded as G6PD deficient (*). It is presumed that he is hemizygous for a G6PD deficiency allele. Any male with a red cell G6PD activity of 30% or more of the normal mean can be regarded as G6PD normal. It is presumed he is hemizygous for a G6PD normal allele.
- **In females:** Any female who has red cell G6PD activity less than 30% of the normal mean or median must be regarded as G6PD deficient. It is presumed that she is either homozygous for a G6PD deficiency allele; or that she has bi-allelic mutations (for instance, one Viangchan allele and one Mahidol allele); or that she is heterozygous for a G6PD deficiency allele, with predominance of a G6PD deficient RBC population. Any female who has a red cell G6PD activity of 80% or more of the normal mean or median can be regarded as 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 predominance of G6PD normal RBC population. Any female with between 30% and 80% of normal G6PD activity must be regarded as intermediate; it is presumed she is heterozygous for a G6PD deficiency allele and a G6PD normal allele.

(*) – All known G6PD variants that are common or polymorphic have a modal value of red cell G6PD activity that is much less than 30% of normal (for instance, the values are about 5–10% for G6PD A- , <1% for G6PD Mediterranean (27) and about <10% for G6PD Mahidol (28)). Thus, in calling G6PD deficient any male with an activity <30%, we have preferred to err on the side of caution, considering also the fact that if the test is carried out in a patient who already has a haemolytic condition, the level of enzyme will be increased.

The ERG also discussed the current conventional diagnostic testing terminology. Under those conventions, a G6PD-deficiency screening device would be “positive” for deficiency when G6PD activity is absent, and “negative” for deficiency when G6PD activity is normal. In order to avoid confusion, the ERG agreed that the terminology should be clearer; therefore, test outcomes should be reported not simply as positive or negative, but as “deficient” or “normal”. These terms, and their relation to the definition of G6PD deficiency above with respect to the 30% level of normal enzyme activity, bear directly on how to estimate sensitivity and specificity of G6PD diagnostics. Tests should provide a G6PD “deficient” result for samples with less than 30% normal activity and a “normal” result for samples with greater than 30% normal activity.

Phenotype terminologies are summarized in Table 1, above.

Qualitative tests for G6PD deficiency

Over the past decades, the fluorescent spot test (FST) has been the qualitative recommended screening test for G6PD deficiency (8). The ERG accepted that novel qualitative G6PD tests should be at least equivalent to FST in diagnostic performance, because this is the commercial test most widely used by professional clinical laboratory, blood banking, and haematology organizations. However, the procedure is expensive and requires laboratory skills, specialized equipment and a cold chain for labile reagents. These characteristics make the test unfeasible for routine use in rural tropical settings, especially in peripheral health-care facilities where most malaria patients live and seek care (9).

More recently, affordable, qualitative POC lateral flow tests that can be performed at or near the site of patient care have become available. These tests generally require whole blood from finger-prick, and can be performed and interpreted by health workers at the bedside or in the field in <30 minutes.

A comparison of the diagnostic performance of available qualitative tests for G6PD deficiency based on published studies is shown in Table 2, below.

Table 2 Diagnostic performance of qualitative tests for G6PD deficiency

Ref	test	specimen	Gold Std	Threshold	# samples	# deficient s	heterozygotes	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%)
3	FST	venous	BIOLAB O SA/	10% genotype	295	42	34	91 (60%)
4	FST	?	Geno-type	All normal by FST	461	27	61	100 (10%) 43 (genotype) All misclassified by FST
5	Binax NOW	venous	Trinity	4.0 U/gHb	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	1 st gen Care Start	venous	Trinity	Lower limit from 174 normal subject (~30% Mean from >4.56 IU/gHb and [Hb] >12 g/ dl	903	97	-	68
8	Care Start	venous	Trinity		456	46(<30%)	-	90 (<10%) 84.8 (<30%)

¹de Niz et al. 2013 (29), ²la Rue et al. 2014 (30), ³Nantakomol et al. 2013 (31), ⁴Eziefula et al. 2014 (32), ⁵Tinley et al. 2010 (33), ⁶Osorio et al. 2014 (34), ⁷Kim et al., 2011 (35) and ⁸von Fricken et al. 2014 (36). Superscripts denote number in table.

Diagnostic performance of CareStart™ G6PD test

Due to the limitations of the FST and the temperature restrictions for use of the FDA-approved Binax Now G6PD test, the evidence review focused on independent evaluations (published and unpublished) of the only commercially available test potentially appropriate for use in tropical *P. vivax* endemic settings, namely CareStart™ G6PD RDT (Access Bio Inc.).^a A common template that would allow comparable assessment of CareStart G6DP RDT performance across unpublished studies was complete by the principal investigators and key study characteristics and results are presented in Tables 4 and 5 (29).

The published studies of the CareStart™ G6PD test available at the time of the meeting were encouraging particularly findings of von Fricken et al. in which the assay had 90% sensitivity for detecting subjects with <10% G6PD activity. However, sensitivity reduced to 84.8% for patients with <30% G6PD activity (36).

Data from unpublished studies evaluating an improved version of the CareStart G6PD RDT compared to both quantitative assays and different brands of FST, produced more encouraging results with laboratory technicians performing the tests in laboratory settings (only one study was performed by lab technicians in the field, Satyagraha ref. #4, in Table 3 and 4).

a. Access Bio has recently launched and CE marked a quantitative Biosensor product, but no published or unpublished reports of independent evaluations were available to review (30, 31, 33).

Table 3 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
Thailand/ G. Bancone* ²	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
		Capillary					100.0	100.0	100.0	100.0		
Thailand/ G. Bancone* ²	R&D Diagnostic	Venous	Lab	technician		28–29	96.0	100.0	100.0	96.3	9–18/150	
		Capillary					100.0	100.0	100.0	100.0		
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
Indonesia/A. Satyagraha* ³	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

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

¹ Based on Roca-Feltre et al. (37)

² Based on Bancone et al. (28)

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

⁴ Study in males only.

Table 4 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 (%) / 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	94.5	36.6	100.0	3.6/419	G6PD Quantitative Trinity Biotech
Thailand / G. Bancone	CareStart v2	Venous	Lab	technician	2 independent readers, if discordant, a third reader	28–29	90.9	97.4	90.0	97.4	-	G6PD Quantitative Trinity Biotech
		Capillary					100.0	82.7	60.6	100.0		
Thailand / G. Bancone	R&D Diagnostic	Venous	Lab	technician		28–29	95.5	97.4	91.3	98.7	-	
		Capillary					100.0	97.4	91.7	100.0		
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
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

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

¹ Based from Roca-Feltre et al. (37)

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

Preferred product characteristics of qualitative point-of-care G6PD RDTs

Review of the performance data for CareStart G6PD RDT prompted discussion on preferred product characteristics for POC G6PD RDTs. Specifically, there was consensus for the following product characteristics:

- >95% sensitive compared to spectrophotometry or equivalent quantitative tests at detecting G6PD enzyme activity levels <30% of normal;
- negative predictive value of >95%, 95% probability that the patient has >30% normal G6PD activity, when the diagnostic test yields a non-deficient result;
- stable at temperatures expected in tropical settings (30–40°C); and
- visual readout that clearly distinguishes between “deficient” and “normal” patients.

G6PD testing and female heterozygotes

As already mentioned, classification of a heterozygote female as G6PD deficient is not straightforward, because the majority of heterozygotes have intermediate levels of G6PD activity. In other words, a qualitative classification of “normal” will include many heterozygotes with intermediate G6PD activity. In light of this issue Baird et al. (38) evaluated diagnostic performance of the FST and CareStart™ G6PD kits using an in vitro copper G6PD inhibition model. The graph in Figure 6 below illustrates the key findings.

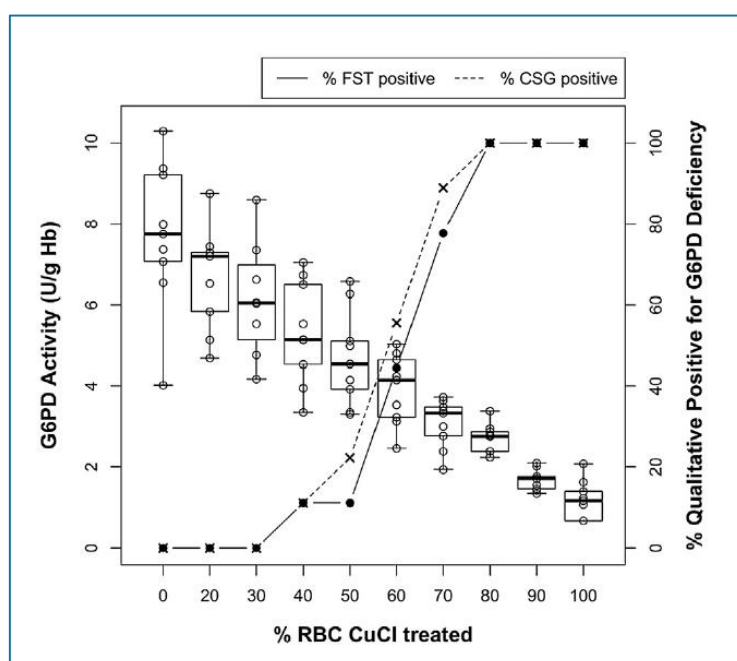


Figure 6 Relationship between net residual enzyme activity in red blood cell suspensions containing increasing proportions of wholly-deficient (copper treated) RBC, along with diagnostic performance of the FST and CareStart™ G6PD kits

From Baird et al. (38)

These data confirm that as the proportion of normal RBCs rises from 30% to 80%, the ability of either kit to result in a “normal” classification increases linearly without a clear cut-off point. In general, at 50% normal RBCs, the probability of being classified as deficient is about 50%. The probability of a “normal” classification increases linearly with increasing net G6PD activity.

Consequences of primaquine dosing in G6PD heterozygotes

Daily dosing for 14 days (at 0.25 mg/kg)

There are few studies evaluating the haematological effects of primaquine in heterozygous G6PD deficient females. Only two published studies (39, 40) reported hemolytic effect of primaquine given to heterozygous females for 14 days as part of a vivax malaria treatment regimen. In one of the studies (40), the subjects were mostly of G6PD Mahidol genotype. In four studies that included heterozygous females with the G6PD A- variant, subjects were administered a single dose of primaquine and the Hb response followed. A heterozygous child with severe anaemia recovered well without requiring blood transfusion. In the other study [39] with a small sample size, the fall in Hb observed by day 7 post-primaquine administration was similar among heterozygous females compared to that among hemi/homozygotes given higher primaquine doses.

In a GSK-sponsored study of tafenoquine (TAF 110027), 4 heterozygous women were treated with 15 mg primaquine base for 14 days and showed a pattern and level of drop of Hb (2.5 g/dL) similar to that observed in all patients with G6PD deficiency (Figure 7 below shows individual haematological parameters). These women had G6PD activity levels ranging between 40% and 60% of normal (J. Green, personal communication).

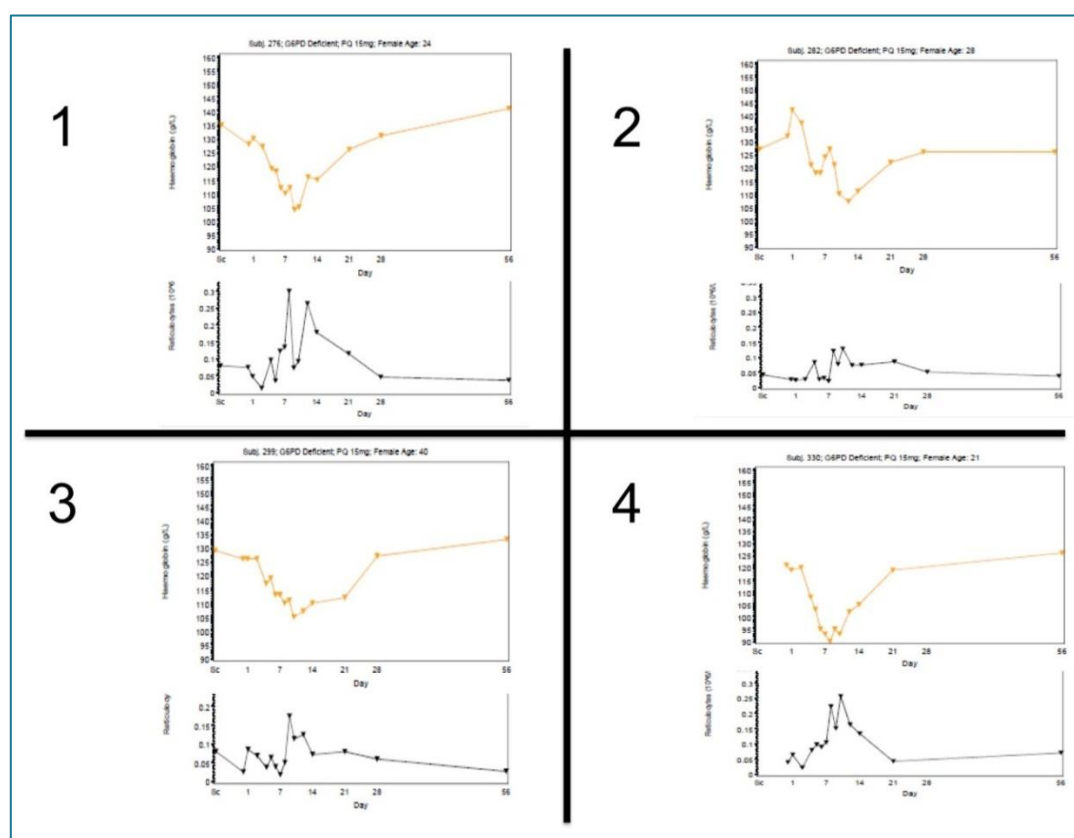


Figure 7 Haemoglobin (orange, above) and reticulocyte (black, below) levels with daily primaquine for 14 days at 0.25 mg/kg/day among four women heterozygous for G6PD deficiency

Courtesy of GSK.

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 pretreatment 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. These findings, in Mahidol variant women having 40% to 60% of normal G6PD activity effectively mirror those in the experiments shown in Figure 8 below, African A - hemizygous men.

No firm conclusions may be drawn regarding extent of hemolysis and risk in broader human populations on the basis of only four subjects. Nonetheless the experiments affirm that female heterozygotes of the “deficient” phenotype (<30% of normal G6PD) will certainly hemolyse following exposure to daily primaquine therapy, even at the lower 0.25 mg/kg dose.

Weekly dosing for 8 weeks (at 0.75 mg/kg)

The ERG considered recent evidence concerning the safety of repeated weekly dose of 45 mg primaquine (0.75 mg/kg) for 8 weeks. This regimen has been recommended by WHO since soon after its description in 1960 as a safe and efficacious regimen. However, it is based on a relatively small number of otherwise healthy volunteers carrying the less severe G6PD A- variant (24). Figure 8 below summarizes some of those findings.

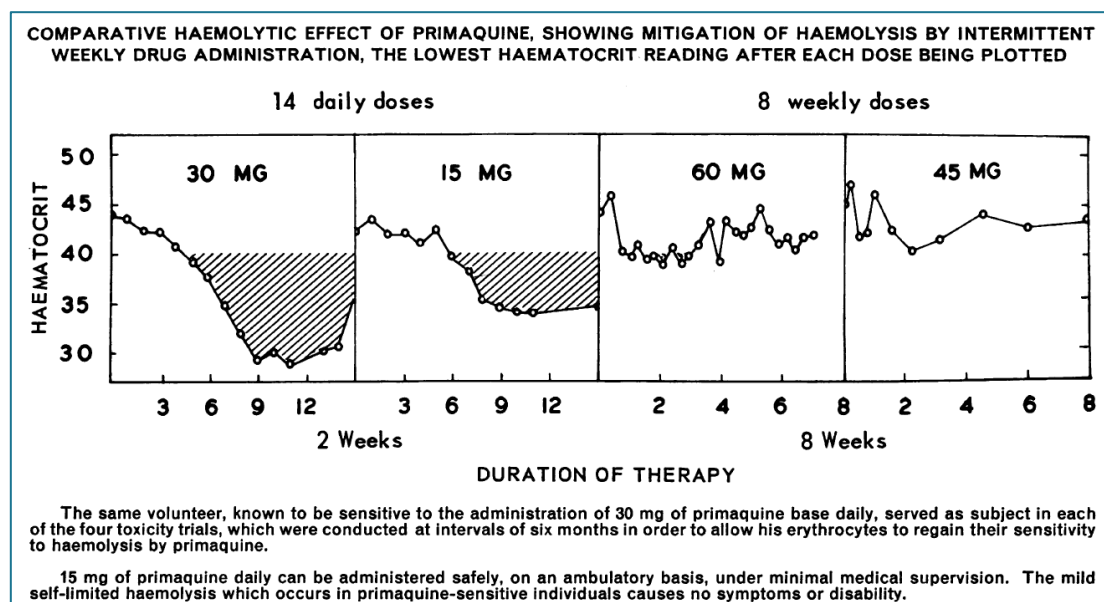


Figure 8 Distinction in haemolytic response between daily versus weekly dosing with primaquine in the same human subject represented by each of the four panels
Reproduced from Alving et al. (24).

In a recent study in Cambodia, the safety of the 0.75 mg/kg weekly dose of primaquine was assessed in hemizygous males carrying the Viangchan variant of G6PD deficiency and suffering acute vivax malaria (Kheng Sim et al., submitted). A total of 75 patients were enrolled aged between five to 63 years. G6PD status was normal in 57 patients and deficient in 18: Canton (n=1) and Viangchan (n=17). Three of the latter were heterozygous females and all other deficient patients were hemizygous males. The variation of Hb concentration over time after treatment with 45 mg primaquine base on a weekly basis is shown in the Figure 9 below.

There was extensive debate on the risk associated with a high dose regimen (even with weekly dosage), and how this can only be safely administered with close monitoring and facilities with

blood transfusion capabilities. Haemoglobin levels in both deficient and normal subjects declined in the two days following therapy, and only slightly more steeply among the deficient. Haemoglobin concentration then levelled and gradually increased, despite doses given again on days 14, 21, 28, 35, 42, and 49 of the study.

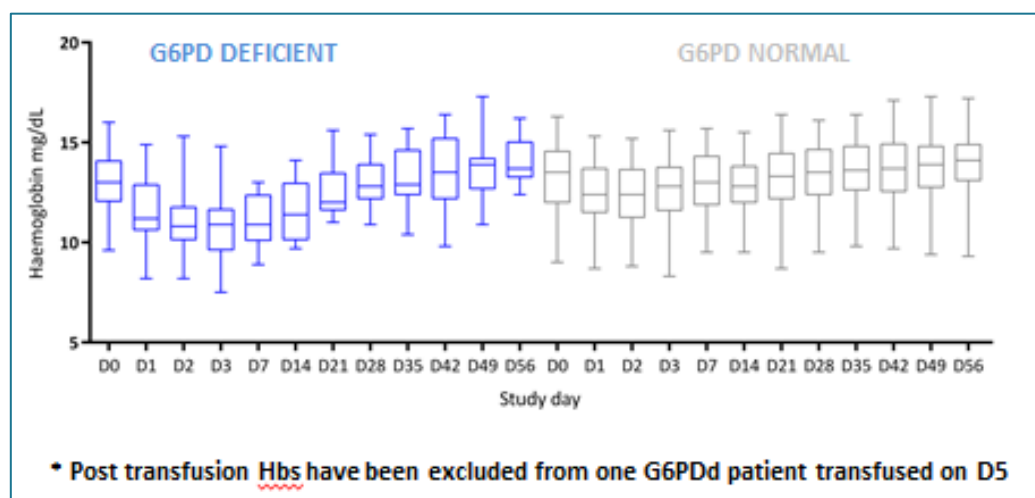


Figure 9 Graphs show responses to single weekly doses of 45 mg primaquine in 18 G6PD deficient (left) and 56 normal (right) male patients with acute *P. vivax* in western Cambodia

Recommendations on G6PD testing and primaquine anti-relapse therapy

After reviewing the evidence presented, and considering the specific questions listed as specific objectives of the meeting, the ERG elaborated the following recommendations for consideration by the Malaria Policy Advisory Committee.

1. G6PD status should be ascertained if possible before administering daily primaquine therapy for 14 days to prevent relapses in patients with confirmed acute *P. vivax* or *P. ovale* infection.
2. G6PD qualitative point-of-care tests to identify G6PD non-deficient patients prior to primaquine administration should be >95% sensitive compared to spectrophotometry or equivalent quantitative tests, stable at temperatures expected in tropical settings (35–40°C) and have a negative predictive value of >95% at G6PD enzyme activity levels <30% of normal.
3. Males who have tested or who have a history of testing normal using a reliable G6PD test should receive standard daily primaquine therapy, as they are not expected to experience harmful adverse drug effects.
4. G6PD qualitative tests will not identify the majority of heterozygous females some of whom may be at risk of developing AHA secondary to primaquine therapy. Therefore, females who test G6PD normal with a qualitative test should only receive daily primaquine therapy if they can be monitored for signs and symptoms of AHA during the first week of treatment.
5. Male or female patients diagnosed with acute *P. vivax* or *P. ovale* malaria should not receive daily primaquine to prevent relapses when they have tested G6PD deficient. However, these patients may receive a weekly dose of 0.75 mg/kg for 8 weeks provided they are under close medical supervision for signs and symptoms of acute hemolytic anaemia during the first 3 weeks of treatment; and provided they have access to health facilities with capacity for safe blood transfusion.
6. If G6PD status is unknown and testing is not available then a decision to prescribe daily primaquine to prevent relapses must be based on a balanced assessment of the following:
 - i) The available data regarding the local prevalence of G6PD deficiency in the population;
 - ii) The capacity to identify and safely monitor and then manage primaquine-induced hemolytic reactions in the treatment setting;
 - iii) The benefits of treatment in terms of expected reduction in number of relapses
7. Patients diagnosed with acute *P. vivax* or *P. ovale* malaria and whose G6PD status is unknown may receive a weekly dose of 0.75 mg/kg for 8 weeks under close monitoring for signs and symptoms of acute hemolytic anaemia during the first 3 weeks of treatment, with access to health facilities with blood transfusion services.

Annex 1 – Listing of the meeting pre-reads

1. Baird, JK *et al.* Noninferiority of Glucose-6-Phosphate Dehydrogenase Deficiency Diagnosis by Point-of-care Rapid Test vs the Laboratory Fluorescent Spot Test Demonstrated by Copper Inhibition in Normal Human Red Blood Cells. (2014) *Translational Research*, online September.
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5. Bancone, G. Diagnostic performance of G6PD POC tests in Thailand.
6. Domingo, G. Overview of performance of point-of-care tests of G6PD deficiency available on the market.
7. Douglas, T. Is it ever ethically acceptable to use primaquine as a hypnozoitocide for vivax malaria without testing for G6PD deficiency?
8. Green, J. Clinical summary of investigations to determine the hemolytic potential of Tafenoquine in G6PD deficient subjects.
9. Howes, R. Distribution of G6PD deficiency and *P. vivax* malaria.
10. Kheng, S *et al.* Tolerability and safety of weekly primaquine against relapse *Plasmodium vivax* in glucose-6-phosphate dehydrogenase deficient and normal Cambodians.
11. Menard, D. Field trial evaluation of the performances of point-of-care tests for testing G6PD deficiency in Cambodia.
12. Mukherjee, M. Influence of individual G6PD genetic variants found in India on severity of hemolysis.
13. Phung Duc, T. Results of diagnostic performance of G6PD POC tests in Vietnam.
14. Recht, J. Primaquine-related hemolysis risk in glucose-6-phosphate dehydrogenase (G6PD) deficient heterozygous females: a review prepared for WHO ERG meeting October 2014.
15. Satyagraha, A. Analysis of G6PD Tests in Indonesia.

Annex 2 – List of participants

Kevin BAIRD (co- rapporteur)
Eijkman Oxford Clinical Research Unit, Jakarta
Indonesia

Germana BANCONE
Shoklo Malaria Research Unit, Mae Sot
Thailand

Gonzalo DOMINGO
PATH, Seattle
USA

Thomas DOUGLAS
Oxford Uehiro Centre for Practical Ethics
University of Oxford, Oxford
United Kingdom

Marcelo FERREIRA
Department of Parasitology, Institute of
Biomedical Sciences
University of Sao Paulo, Sao Paulo
Brazil

Paul GARNER
Liverpool School of Tropical Medicine,
Liverpool
United Kingdom

Rosalind HOWES
Spatial Ecology and Epidemiology Group
University of Oxford, Oxford
United Kingdom

Sim KHENG
Department of Communicable Disease Control
Ministry of Health, Phnom Penh
Cambodia

Marcus VG LACERDA
Tropical Medicine Foundation Dr Heitor Vieira
Dourado, Manaus
Brazil

Lucio LUZZATTO (chairperson)
Istituto Toscano Tumori, Florence
Italy

James McCARTHY
Clinical Tropical Medicine Laboratory
Queensland Institute of Medical Research
Berghofer, Herston
Australia

Didier MENARD
Malaria Molecular Epidemiology Unit
Institut Pasteur, Phnom Penh
Cambodia

Malay MUKHERJEE
National Institute of Immunohematology,
Mumbai
India

Francois NOSTEN
Shoklo Malaria Research Unit, Mae Sot
Thailand

Thuan PHUNG DUC (unable to attend but
shared result as pre-reads of the meeting)
Oxford University Clinical Research Unit Vietnam
(OUCRU)
Hospital for Tropical Diseases, Ho Chi Minh City
Viet Nam

Judith RECHT
Independent Consultant, Recife
Brazil

Ari SATYAGRAHA (co- rapporteur)
Eijkman Institute for Molecular Biology, Jakarta
Indonesia

Walter R. TAYLOR
Mahidol Oxford Tropical Medicine Research
Unit
Mahidol University, Bangkok
Thailand

Observers

Janice CULPEPPER
Bill & Melinda Gates Foundation, Seattle
USA

Penny Grewal DAUMERIE
Medicine for Malaria Venture, Geneva
Switzerland

Stephan DUPARC
Medicines for Malaria Venture, Geneva
Switzerland

Justin GREEN
GlaxoSmithKline, Uxbridge
United Kingdom

WHO secretariat

Andrea BOSMAN, Global Malaria Programme

Jane CUNNINGHAM, Global Malaria Programme

Peter OLUMESE, Global Malaria Programme

John REEDER, Acting Director, Global Malaria Programme and Director, Special Programme for Research and Training in Tropical Diseases

Pascal RINGWALD, Global Malaria Programme

Silvia SCHWARTE, Global Malaria Programme

Lasse VESTERGAARD, WHO Regional Office for WPRO (via Skype)

Marian WARSAME, Global Malaria Programme

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