G6PD deficiency in Latin America: systematic review on prevalence and variants

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Plasmodium vivax *radical cure requires the use of primaquine (PQ), a drug that induces haemolysis in glucose-6-phosphate dehydrogenase deficient (G6PDd) individuals, which further hampers malaria control efforts. The aim of this work was to study the G6PDd prevalence and variants in Latin America (LA) and the Caribbean region. A systematic search of the published literature was undertaken in August 2013. Bibliographies of manuscripts were also searched and additional references were identified. Low prevalence rates of G6PDd were documented in Argentina, Bolivia, Mexico, Peru and Uruguay, but studies from Curaçao, Ecuador, Jamaica, Saint Lucia, Suriname and Trinidad, as well as some surveys carried out in areas of Brazil, Colombia and Cuba, have shown a high prevalence (> 10%) of G6PDd. The G6PD A-^{202A} mutation was the variant most broadly distributed across LA and was identified in 81.1% of the deficient individuals surveyed. G6PDd is a frequent phenomenon in LA, although certain Amerindian populations may not be affected, suggesting that PQ could be safely used in these specific populations. Population-wide use of PQ as part of malaria elimination strategies in LA cannot be supported unless a rapid, accurate and field-deployable G6PDd diagnostic test is made available.*

Key words: G6PD deficiency - malaria control - *Plasmodium vivax* - primaquine - haemolysis - Amerindians

Glucose-6-phosphate dehydrogenase (G6PD) is a cytoplasmic enzyme. The major function of G6PD is the prevention of oxidative damage to cells by promoting detoxification of free radicals. This metabolic pathway involves the production of nicotinamide adenine dinucleotide phosphate (NADPH), which participates in the glutathione (GSH) cycle, protecting the cell against hydrogen peroxide-induced damage and assuring an oxidative balance profile within the cell (Cappellini & Fiorelli 2008). The G6PD gene presents an X-linked pattern (Beutler 1994), where hemizygous males and homozygous females are deficient and heterozygous females may or may not be deficient because of mosaicism. Despite the majority of G6PD deficient (G6PDd) individuals being asymptomatic, some present haemolytic incidents that may be triggered by intrinsic or extrinsic stressors, e.g., diabetic ketoacidosis, drugs, pathogens or types of food, causing syndromes such as acute haemolytic anaemia (AHA), neonatal jaundice (NNJ) and congenital non-spherocytic haemolytic anaemia, with the resulting manifestations depending on the amount of oxidative stress experienced in the cells and the levels of enzyme activity (Mason et al. 2007).

G6PDd is found worldwide with varying frequencies depending on the region and ethnic group. The overall G6PDd allele frequency across malaria endemic countries is estimated to be 8%, corresponding to approximately 220 million males and 133 million females (Howes et al. 2012). Variable enzyme activity levels have been measured among deficient individuals based on the diversity of mutations. The World Health Organization (WHO) has classifies G6PDd according to enzyme activity, ranging from ≤ 1% to over 150%, classes I-V, respectively, in which classes I, II and III are considered at risk for haemolytic events (WHO 1989). The highest G6PDd prevalence has been found to occur in sub-Saharan Africa and the Arabian Peninsula (Howes et al. 2012). Recent data found 186 known G6PD genotypic variants, most of them resulting in phenotypes that are asymptomatic until exposed to haemolytic triggers (Minucci et al. 2012). These G6PD variants are heterogeneously spread among different countries, presenting distinct region-specific distributions (Howes et al. 2013), which could be related to specific routes of human migration. Among all the known deficiencies, the G6PD A- and the

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G6PD Mediterranean variants have been reported to be detected more frequently in certain populations and are the variants primarily responsible for the occurrence of haemolytic events (Frank 2005). The G6PD A- variant is the most predominant allele in the African continent, whereas the G6PD Mediterranean allele is more frequent in Western Asia (Howes et al. 2013).

Malaria in humans is predominantly caused by two Plasmodia species, *Plasmodium falciparum* and *Plasmodium vivax* and is estimated to be spread over more than 90 countries, putting nearly 3.3 billion people at risk of disease (Guerra et al. 2010, WHO 2012a). The prevalence of G6PDd across countries was found to have a good correlation with those where, historically, malaria transmission has occurred (Howes et al. 2012). The explanation for this association has been that G6PDd is associated with protection against *P. falciparum* (Mockenhaupt et al. 2003, Clark et al. 2009) and *P. vivax* infections (Leslie et al. 2010, Santana et al. 2013). The mechanism conferring resistance in G6PDd subjects may be related to an impaired antioxidant defence in ring-stage parasitised red cells, which could lead to membrane damage, triggering increased removal of infected cells by phagocytosis before parasite maturation to the trophozoite and schizont stages (Ruwende et al. 1995). G6PDd is also thought to be a protection factor against severe manifestations of malaria, although studies regarding which individuals, hemizygous males or heterozygous females, may be protected present discrepancies (Ruwende et al. 1995, Guindo et al. 2007).

In a context where the international community has committed to a renewed malaria eradication agenda (malERA 2011), many countries have been planning to reduce transmission or eliminate malaria, making the development and deployment of cost-effective strategies extremely necessary (Das & Horton 2010). Primaquine (PQ), an 8-aminoquinoline, is currently the only Food and Drug Administration approved drug recommended to treat hepatic stages of the *P. vivax*. The WHO recommends that PQ should be used in radical cure for *P. vivax* malaria (0.5 mg/kg/d for 14 days) (WHO 2010) or as a gametocytocidal agent in uncomplicated *P. falciparum* infections (0.25 mg/kg in a single dose) (WHO 2012b). The use of PQ or other 8-aminoquinolines such as tafenoquine (undergoing Phase III clinical trials) in G6PDd individuals presents serious risks, as these compounds may induce life-threatening haemolytic events, the intensities of which depends on the individual's enzyme activity profile (Llanos-Cuentas et al. 2013, von Seidlein et al. 2013). The WHO recommends that in mild-to-moderate G6PDd, PQ (0.75 mg base/kg) should be given once a week over eight weeks, while in severe G6PDd patients, PQ is contraindicated (WHO 2010).

Geographically, *P. vivax* is more widely distributed than *P. falciparum*, with the former species having a potential to cause morbidity and mortality amongst the 2.85 billion people living at risk of infection, most of them in Central and Southeast Asia and Latin America (LA) (Guerra et al. 2010). In LA and the Caribbean, only Chile, Uruguay, Cuba, Bahamas, Jamaica and other small countries in the Caribbean are now considered malaria-free, while Mexico, Haiti, Costa Rica, El Salvador, Panama, Argentina and Paraguay have been classified as malaria-eliminating countries. The remaining countries are still in the phase of controlling transmission (WHO 2012a). Predominance of *P. vivax* malaria in these countries is especially relevant given that radical cure requires the use of PQ, implying a risk of haemolysis and its adverse consequences in G6PDd individuals (Beutler & Duparc 2007). The public health consequences of this condition deserve special attention because of the impossibility of using PQ in regions where there is a high prevalence of this deficiency, further hampering transmission control efforts for this parasite species (White 2008, Baird & Surjadjaja 2011). Despite the clinical and epidemiological significance of the interaction between G6PDd and malaria, the prevalence of G6PDd and the extent of its clinical consequences have not been properly measured in LA populations.

In Brazil and other LA countries facing malaria as a public health problem, the treatment recommendation for *P. vivax* includes a full-course of PQ with no routine G6PDd screening. This short-sighted policy leads to the prescription of potentially haemolytic drugs, putting G6PDd individuals at risk of serious complications (Lacerda et al. 2012b, Monteiro et al. 2014). We therefore reviewed the existing literature to estimate the prevalence of G6PDd in LA and to identify the most frequent variants that affect this population, highlighting areas where more research is necessary in the region.

MATERIALS AND METHODS

A systematic review was performed to analyse the available published data on G6PDd prevalence, phenotypes and mutations in LA and the Caribbean. A broad free text search was made using a previously published search strategy (Monteiro et al. 2014). Potentially relevant papers in all languages were accessed from MEDLINE and LILACS for a review of the full texts. Additional articles were obtained through citation tracking of reviews/opinion articles and original papers. To identify relevant papers, two independent reviewers examined titles, abstracts and full texts of the retrieved studies. Existing databases (Arends 1966, Fonseca et al. 2005, Howes et al. 2012, 2013) were reviewed for any additional sources.

For G6PDd prevalence analysis, results of phenotyping tests from surveys in males were used. Potentially biased samples, including malaria patients, ethnically selected samples, individuals with a previous history of haemolytic anaemia, new-borns presenting jaundice and surveys based only on the search of specific genotypes were excluded because they tend to underestimate the real G6PDd prevalence (Howes et al. 2012). LA or Caribbean population migrants living in other continents were also excluded. Only surveys with precise information of the study location were included. Data on location of the study (country and locality), number of people tested, sex, number of G6PDd subjects, ethnicity, G6PD phenotypes/mutations found, relative prevalence of each variant and authorship information were extracted directly from the full length articles to structured Tables and Figures containing all the descriptive variables.

For G6PDd variant analysis, two types of studies were included: (i) representative community surveys on

G6PDd prevalence assessed by gel electrophoresis and/or genotyping methods and (ii) non-representative surveys, including all types of studies and populations. Thus, to build a G6PDd database of the variant diversity in LA and the Caribbean, both types of papers were included. Classification of the variants was made according to a review published previously (Minucci et al. 2012) and according to information given by the original articles.

A map was created with the software ArcMap 10.1 in ArcGIS 10.1 (ESRI, USA) using estimates of the crude average prevalence for males. Separate male analysis and estimates for the map values were performed because phenotypic expression is more reliable, thus rendering male estimates more suitable for cross-locality comparisons (Howes et al. 2012). Spatial interpolation of G6PD prevalence was mapped using 70 representative community surveys corresponding to 103 study sites where males had been sampled. Duplicate studies performed at the same locations were excluded and the study with the bigger sample size was retained. Several different interpolation methods were tried, including ordinary kriging, Kernel Interpolation with Barriers and Inverse Distance Weighting (IDW). The latter was selected as it produces a contour surface that best represents broad zones where different G6PDd values may be predicted. IDW is a technique that determines cell values using a linearly weighted combination of a set of sample points. The weight is a function of inverse distance and the method assumes that the variable being mapped decreases in influence with distance from its sampled location (ArcMap 10.1 documentation). For this analysis, the output cell size was set to 0.33, a power value of 2 was used, the search neighbourhood was set to smooth circular with a 30.15 radius and a smoothing factor of 0.2 was used. The sample size was used as a weight variable. No features were used as barriers.

RESULTS AND DISCUSSION

The MEDLINE search generated 487 papers and the LILACS search generated 140 papers. After assessing the inclusion criteria, 70 original papers with representative community surveys on G6PDd prevalence in males from 18 LA and Caribbean countries were found (Supplementary data). This systematic review also revealed 24 publications regarding G6PDd prevalence among Amerindian populations (16 publications from the original search and 8 from after reading these primary references).

Moreover, 71 appropriate original papers regarding G6PD variant characterisation were retrieved. Thirty-two additional articles were obtained through citation tracking of reviews/opinion articles and original papers. Of these 103 articles, 41 reported data from representative community surveys with G6PDd typing in males (28 using gel electrophoresis phenotyping and 13 using genotyping methods) (Supplementary data).

Prevalence estimates of G6PDd in LA and Caribbean - Overall numbers of individuals sampled were 63,716 males and 12,868 females (Table I). Considering only malaria endemic countries, 53,399 males and 10,079 females were tested. Brazil was the country with the largest number of males tested (n = 28,671; 53.7% of the male population tested in malaria endemic countries), followed by Mexico (n = 13,126; 24.6%). Low prevalence rates were recorded from Argentina, Bolivia, Mexico, Peru and Uruguay. Studies performed in Curaçao, Ecuador, Jamaica, Saint Lucia, Suriname and Trinidad, as well as some surveys carried out in areas of Brazil, Colombia and Cuba, have shown a high prevalence (> 10%) of G6PDd. Studies were unevenly distributed, with some malaria endemic countries, including Belize, Guatemala, Nicaragua, Panama, Guyana, French Guiana and Paraguay, not contributing any data.

Mapping showed large swathes in LA and Caribbean countries where G6PDd was predicted to have prevalence ≤ 2%, namely in Mexico, Guatemala, Peru, Bolivia, Uruguay, Chile and Argentina (Fig. 1). Relative to these countries, prevalence was higher in the Caribbean islands, French Guiana, Suriname, Guyana, northwestern Venezuela and Pacific coastal regions of Colombia and Ecuador, where the majority of the estimated areas of prevalence above 10% were located. In the Amazon Region, prevalence estimates ranged from 4% in the southern areas to 10% in the Guiana borders. Prevalence in the northern Amazonian area is most likely overestimated due to the high G6PDd prevalence in Guyana and Suriname in the mapping model.

This map presents estimates of sub-national variations of G6PDd prevalence across LA and Caribbean countries. Recently, a global map presenting data at sub-national levels using the same inclusion and exclusion criteria for retrieving articles was published (Howes et al. 2012). These authors mapped LA and Caribbean G6PDd based on 34 published articles. In the current review, by searching in LA specific databases and not applying any language restrictions, we were able to increase the number of usable publications to 70 articles. This higher sensitivity (great number of literature published regionally retrieved from LILACS database) most likely adds

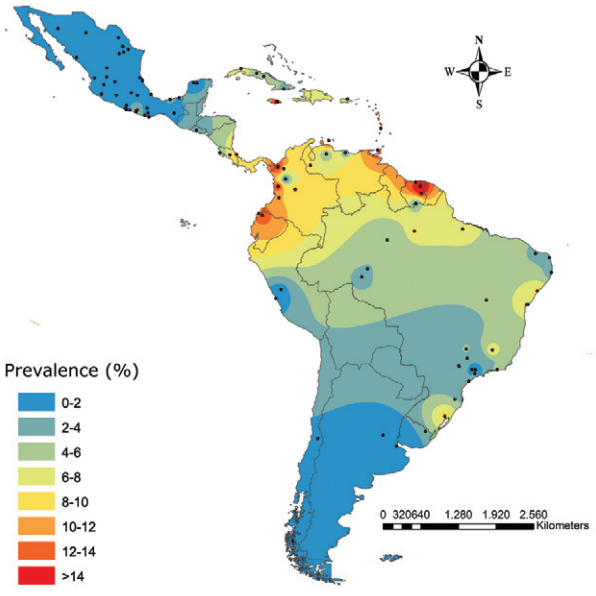

Fig. 1: map of estimated prevalence of glucose-6-phosphate dehydrogenase deficient across Latin America and the Caribbean countries.

TABLE I

Summary of the results of surveys on glucose-6-phosphate dehydrogenase deficient prevalence in Latin America and the Caribbean

Country	Male (n)	Prevalence range among studies (%)	Female (n)	Prevalence range among studies (%)	Population not discriminated by sex[a] (n)	Prevalence range among studies (%)
Argentina	4,437	0.3-0.5	-	-	4,642	1.1
Bolivia	335	0.7-1.5	200	0	-	-
Brazil	28,671	0-12.9	6,709	0-13.6	9,801	1.1-13.3
Colombia	988	1.4-15.4	-	-	611	3.1-12.7
Costa Rica	1,371	0.4-12.6	1,683	0-7.8	1,628	0.4-5.4
Cuba	7,957	0.6-16.1	1,813	0.7-10.2	500	3.6
Curaçao	573	14	213	10.3	-	-
El Salvador	778	2.4	416	6	370	0.5
Ecuador	1,173	9.3-12.8	-	-	-	-
Jamaica	976	13.5-22.6	524	4.1-28.3	-	-
Mexico	13,126	0-12.7	488	0-0.9	2,464	0-0.6
Peru	640	0-0.7	-	-	-	-
Puerto Rico	56	5.4	143	2.8	-	-
Saint Lucia	427	14.8	-	-	-	-
Suriname	1,507	3.2-20.2	422	1.4	-	-
Trinidad	328	13.4	-	-	-	-
Uruguay	-	-	96	0	144	2.1
Venezuela	373	0-3.5	161	1.8-2.9	112	0
Total	63,716	0-22.6	12,868	0-28.3	20,272	0-13.3

a: from studies conducted with male and female individuals without gender-specific prevalence.

more accuracy in the spatial distribution of G6PDd in this continent. The geographic patterns presented here corroborate the findings of a previous study by Howes et al. (2012), which showed lower G6PDd frequencies in Mexico, Argentina, Bolivia and Peru. However, a higher G6PDd prevalence was found in the Caribbean islands, Guianas, Pacific coastal regions of Colombia and Ecuador and part of the Brazilian Atlantic Coast compared to the previous study. These regions coincide with the areas that received the greatest contribution of African immigrants, particularly as part of the slave trade (Franco 1979).

G6PDd variants in LA and Caribbean - LA studies concerning the characterisation of the G6PDd were mostly conducted using gel electrophoresis and mainly carried out before 1990. As shown in Fig. 2A, the results from biochemical characterisations performed after representative community surveys on G6PDd prevalence revealed a broad geographic range of the African variant, which was recorded in 28 studies carried out in nine countries. Among 10,385 males tested in these studies, 518 G6PDd individuals were identified and further typed as carriers of the African (463 males; 89.4%), Mediterranean (41 males in 5 countries; 7.9%) or Seattle (1 male from Brazil; 0.2%) variants. Samples from 13 individuals were not conclusively characterised by gel electrophoresis. In addition to G6PDd variant characterisation using samples from community surveys, 35 additional biochemical variants were recorded and published as case reports (see database presented in Table II). These publications are generally descriptions of putative new G6PD variants in people presenting non-spherocytic anaemia or episodes of haemolysis.

Fig. 2B presents the results from characterisation of G6PD variants at the DNA level performed after 13 community surveys on G6PDd prevalence from five countries. It showed that the G6PD encoded by the *G6PD A-^{202A}* mutation is the most broadly distributed across LA and was identified in 81.1% of deficient individuals surveyed in the continent (687 of 847 total G6PDd males surveyed). Other variants identified included *G6PD A-^{968G}* (59 males; 7%), G6PD Mediterranean (19; 2.2%), Santamaria (16; 1.9%), Seattle (16; 1.9%), Belém (3; 0.3%) and Chatham (2; 0.2%). Some other rare variants were recorded from Belém, in the Eastern Brazilian Amazon (Hamel et al. 2002). Thirty-four individuals were not typed because some investigations do not perform total G6PD gene sequencing, thus indicating that new mutations possibly exist in this continent. In Campinas, Brazil, nine out of the 74 deficient individuals were found to carry the G6PD A-^{202A} mutation (12.2%) (Saad et al. 1995). A community screening survey showed an estimated prevalence of 6% for the G6PD A-^{202A} in Acrelândia, Brazil (Cardoso et al. 2012).

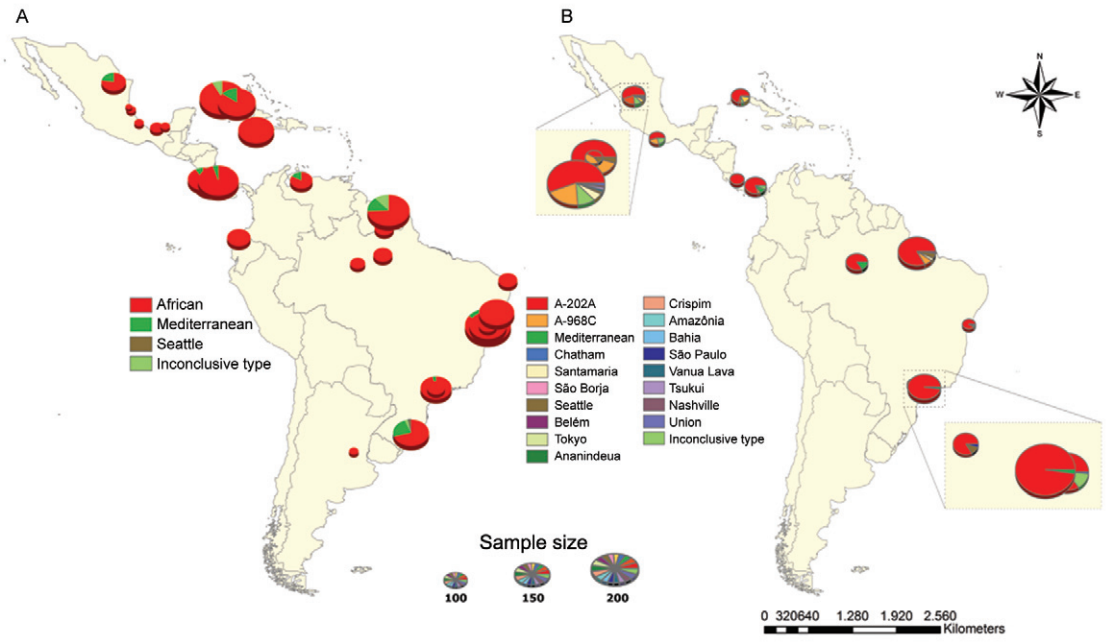

Fig. 2: spatial distribution of glucose-6-phosphate dehydrogenase deficient variants characterised by gel electrophoresis (A) and molecular analysis (B) in Latin American and Caribbean community-level studies. Sample size is reflected in the size of the pie charts.

TABLE II

Glucose-6-phosphate dehydrogenase phenotypes found in Latin America and the Caribbean

Name	Class	Country	References	Frequency (%)
A-	III	Brazil	Saldanha et al. (1969), Azevedo and Azevedo (1974), Azevêdo et al. (1980, 1981), Weimer et al. (1981, 1991, 1998), Franco et al. (1982), Conceição et al. (1987), Santos et al. (1987), Schneider et al. (1987), Garlipp and Ramalho (1988), Saad and Costa (1992), Bortolini et al. (1997), Silva et al. (2004), Santana et al. (2009), Iglessias (2009)	89.4
		Costa Rica	Sáenz et al. (1985, 1986), Chaves et al. (1988), Madrigal et al. (1990)	
		Cuba	González et al. (1975), Hidalgo et al. (1987)	
		Ecuador	Martínez-Labarga et al. (1999)	
		Jamaica	Gibbs et al. (1972)	
		Mexico	Lisker et al. (1966, 1969, 1981, 1988, 1990)	
		Suriname	Geerdink et al. (1974)	
		Venezuela	Boada and Yates (1979), Bortolini et al. (1998)	
		Uruguay	Weimer et al. (1998)	
Attica	III	Puerto Rico	McCurdy et al. (1973)	Rare
Aymara	None	Chile	Ferrell et al. (1980)	Rare
Carapicuíba	II/III	Brazil	Barretto and Nonoyama (1991)	Rare
Castilla	III	Mexico	Lisker et al. (1977)	
Caujerí	III	Cuba	Gutiérrez et al. (1987)	Rare
Chiapas	II	Mexico	Lisker et al. (1978)	Rare
Chicago	II	Puerto Rico	McCurdy et al. (1973)	
Cuiabá	IV	Brazil	Barretto and Nonoyama (1987)	Rare
Distrito Federal	III	Mexico	Lisker et al. (1981)	

Name	Class	Country	References	Frequency (%)
El Morro	III	Puerto Rico	McCurdy et al. (1973)	Rare
Farroupilha	II/III	Brazil	Weimer et al. (1998)	Rare
Guadalajara	I	Mexico	Vaca et al. (1982)	Rare
Guantánamo	III	Cuba	Gutiérrez et al. (1987)	Rare
Guaíba	IV	Brazil	Weimer et al. (1981, 1998)	Rare
Jalisco	III	Mexico	Vaca et al. (1985)	Rare
Lages	III	Brazil	Weimer et al. (1998)	Rare
Laguna	III	Brazil	Weimer et al. (1984, 1998)	Rare
La Habana	III	Cuba	González et al. (1980)	Rare
Mediterranean	II	Brazil	Azevedo and Azevedo (1974), Azevêdo et al. (1980), Weimer et al. (1981, 1998), Franco et al. (1982), González-González and Calcines (1986), Garlipp and Ramalho (1988), Santana et al. (2009)	7.9
		Costa Rica	Sáenz et al. (1985, 1986), Chaves et al. (1988), Madrigal et al. (1990)	
		Cuba	González-González and Calcines (1986), Hidalgo et al. (1987)	
		Mexico	Lisker et al. (1976)	
		Venezuela	Boada and Yates (1979)	
Mexico City	IV	Mexico	Lisker et al. (1972)	Rare
Minas Gerais	IV	Brazil	Azevedo and Yoshida (1969), Hutz et al. (1977), Weimer et al. (1981, 1998)	Rare
Morelia	IV	Mexico	Vaca et al. (1985)	Rare
Nashville Anaheim	I	Brazil	Weimer et al. (1998)	Rare
Porto Alegre	IV	Brazil	Hutz et al. (1977), Weimer et al. (1981, 1998)	Rare
Puerto Limón	I	Costa Rica	Elizondo et al. (1982)	Rare
Puerto Rico	III	Puerto Rico	McCurdy et al. (1973)	Rare
San José	III	Costa Rica	Castro and Snyder (1974)	Rare
San Juan	III	Puerto Rico	McCurdy et al. (1973)	Rare
Santa Clara	III	Cuba	Gonzalez et al. (1990)	Rare
Santamaría	II	Costa Rica	Sáenz et al. (1984)	Rare
São Borja	IV	Brazil	Weimer et al. (1998)	Rare
São Paulo	IV	Brazil	Barretto (1983)	Rare
Seattle/Athens-like	III	Brazil	McCurdy et al. (1973), Hutz et al. (1977), Weimer et al. (1981, 1998)	0.2
Tepic	III	Mexico	Lisker et al. (1985)	
Trinacria	III	Mexico	Vaca et al. (1985)	Rare
Varadero	I	Cuba	Estrada et al. (1982)	Rare
Villa Clara	III	Cuba	Gonzalez et al. (1990)	Rare

Tepic, Castilla, Distrito Federal phenotypes were identified as A- (202G→A, 376A→G) *a posteriori* according to genetic characterisation (Beutler et al. 1991a). Frequency was retrieved from representative community surveys in males. A rare variant has a prevalence of less than 0.1%.

This systematic review led to the creation of a database of G6PD mutations found in LA and the Caribbean (Table III). Thirty works from seven countries presented data on the enzyme characterisation of G6PD variants at the DNA level. To date, 38 G6PDd mutations have been reported in LA: among these, 30 (78.9%) are single nucleotide substitutions, seven (18.4%) are multiple mutations (2 or more substitutions) and one (2.6%) is a deletion. Eleven variants were identified in class I G6PDd and 27 were classified in classes II or III G6PDd. Twenty-three novel mutations were described in LA.

G6PD characterisation at the DNA level was performed with samples collected from 13 community surveys on G6PDd prevalence from only five countries. This scarcity of data constitutes a major limitation for a more reliable understanding of G6PDd epidemiology

TABLE III

Database of glucose-6-phosphate dehydrogenase mutations found in Latin America and the Caribbean

Name (cDNA nucleotide substitution)	Class	Country	References	Frequency (%)
A- (202G→A, 376A→G)	III	Brazil	Weimer et al. (1993), Saad et al. (1995, 1997b), Compri et al. (2000), Hamel et al. (2002), Saad and Costa (2003), Agudelo-Flórez et al. (2004), Castro et al. (2007), Moura-Neto et al. (2008), Oliveira et al. (2009), Mezzacappa et al. (2010), Cardoso et al. (2012), Santana et al. (2013)	81.1
		Costa Rica	Beutler et al. (1991b)	
		Cuba	del Cueto (2008)	
		Mexico	Beutler et al. (1991a, b), Medina et al. (1995, 1997), Arámbula et al. (2000), Vaca et al. (2002)	
		Panamá	Cossio-Gurrola et al. (2010)	
A- (376A→G, 968T→C)	III	Brazil	Weimer et al. (1993), Hamel et al. (2002), Castro et al. (2007)	7
		Cuba	del Cueto (2008)	
		Mexico	Medina et al. (1995, 1997), Arámbula et al. (2000), Vaca et al. (2002)	
		Panama	Cossio-Gurrola et al. (2010)	
Amazônia (185C→A)	II	Brazil	Hamel et al. (2002)	Rare
Ananindeua (376A→G, 871G→A)	II	Brazil	Hamel et al. (2002)	Rare
Asahi (202G→A)	III	Brazil	Moura-Neto et al. (2008)	Rare
Bahia 1 (197T→A, 202G→A)	III	Brazil	Moura-Neto et al. (2008)	Rare
Bahia 2 (197T→A, 202G→A, 376A→G)	III	Brazil	Moura-Neto et al. (2008)	Rare
Belém (409C→T)	II	Brazil	Hamel et al. (2002)	0.3
Buenos Aires (1465C→T)	I	Argentina	Minucci et al. (2008)	Rare
Campinas (1463G→T)	I	Brazil	Baronciani et al. (1993)	Rare
Calvo Mackenna (1138A→G)	I	Chile	Dal Borgo et al. (2000)	Rare
Chatham (1003G→A)	II	Brazil	Saad et al. (1997b), Compri et al. (2000)	0.2
Crispim (375G→T, 379G→T, 383T→C, 384C→T)	II	Brazil	Hamel et al. (2002)	Rare
Durham (713A→G)	I	Mexico	Vaca et al. (2003)	Rare
Farroupilha (977C→A)	II/III	Brazil	Weimer et al. (1998)	Rare
Guadalajara (1159C→T)	I	Mexico	Beutler et al. (1992), Medina et al. (1997), Vaca et al. (2002)	Rare
Lages (40G→A)	III	Brazil	Weimer et al. (1998)	Rare

Name (cDNA nucleotide substitution)	Class	Country	References	Frequency (%)
Mediterranean (563C→T)	II	Brazil	Weimer et al. (1993), Saad et al. (1997b), Santana et al. (2013)	2.2
		Panama	de Gurrola et al. (2008), Cossio-Gurrola et al. (2010)	
Mexico City (680G→A)	IV	Mexico	Beutler et al. (1992), Medina et al. (1997), Vaca et al. (2002)	Rare
Nashville (Anaheim) (1178G→A)	I	Brazil	Weimer et al. (1993)	Rare
		Mexico	Vaca et al. (2002)	Rare
Puerto Limón (1192G→A)	I	Costa Rica	Beutler et al. (1991b)	Rare
Santamaría (376A→G, 542A→T)	II	Brazil	Hamel et al. (2002)	1.9
		Costa Rica	Beutler et al. (1991b)	
		Cuba	del Cueto (2008)	
		Mexico	Vaca et al. (2002)	
Santiago (593G→C)	I	Chile	Beutler et al. (1992), Dal Borgo et al. (2000)	Rare
Santiago de Cuba (1339G→A)	I	Cuba	Vulliamy et al. (1988)	Rare
São Borja (337G→A)	IV	Brazil	Weimer et al. (1993)	Rare
		Cuba	del Cueto (2008)	
San Luís Potosí (376A→T)	II	Mexico	Vaca et al. (2003)	Rare
São Paulo (660C→G)	IV	Brazil	Oliveira et al. (2009)	Rare
Seattle/Athens-like (844G→C)	III	Brazil	Weimer et al. (1993), Hamel et al. (2002), Oliveira et al. (2009)	1.9
		Mexico	Medina et al. (1995, 1997), Arámbula et al. (2000), Vaca et al. (2002)	
Sumaré (1292T→G)	I	Brazil	Saad et al. (1997a, b), Saad and Costa (2003)	Rare
Tokyo (1246G→A)	II	Brazil	Hamel et al. (2002)	Rare
Tsukui (561_563del)	I	Mexico	Vaca et al. (2002)	Rare
Union (1360C→T)	II	Mexico	Vaca et al. (2002)	Rare
Valladolid (406C→T)	II	Mexico	Vaca et al. (2003)	Rare
Vanua Lava (383T→C)	II	Mexico	Vaca et al. (2002)	Rare
Veracruz (1094G→A)	II	Mexico	Vaca et al. (2003)	Rare
Viangchan (871G→A)	II	Mexico	Vaca et al. (2003)	Rare
Yucatán (1285A→G)	II	Mexico	Vaca et al. (2003)	Rare
Zacatecas (770G→T)	II	Mexico	Vaca et al. (2003)	Rare

frequency was retrieved from representative community surveys in males. A rare variant has a prevalence of less than 0.1%.

in LA. Biochemical methods strongly suggested the predominance of the African variant across this continent, in agreement with other genotyping studies from the same areas, suggesting the reasonable reliability of gel electrophoresis as a diagnostic tool for G6PD enzyme variants.

Investigation of different PQ regimens must also include appropriate measures of tolerability and be able to quantify the risk of severe adverse events, especially in patients with deficient variants of G6PD. However, only a few publications have addressed the correlation between G6PD variants and clinical presentations in LA. Out of the 30 articles reporting G6PDd at the DNA level (Table III), 11 referred to reports of rare class I mutations associated with chronic non-spherocytic haemolytic anaemia. Anaemia was recorded in a case report focusing on one male G6PD A-^{202A} mutation carrier (Agudelo-Flórez et al. 2004). Mild haemolysis was observed in a girl with both G6PD Sumaré (class I variant) and G6PD A- alleles (Saad & Costa 2003). One Brazilian publication presents three male asymptomatic carriers of the Mediterranean, São Borja and Seattle mutations and one carrier of the Anaheim mutation with a history of NNJ, several hospitalisation episodes and severe anaemia (Weimer et al. 1993). None of these mentioned case reports involved treatment with PQ.

The severity of particular G6PD mutations and their prevalence in a population determine the risk of individual (and eventually population-wide) administration of PQ. For example, G6PDd is common among people of African origin, but the enzyme deficiency is relatively mild (A- variant) with most having greater than 10% of the wild-type enzyme activity (Beutler 1991). Patients with the G6PD A- variant are relatively resistant to serious reactions to PQ-induced haemolysis (Dern et al. 1954); G6PD A- individuals dosed with daily PQ for four months have been observed to typically recover from relatively shallow AHA within about three weeks, despite continued daily dosing (Dern et al. 1954, Alving et al. 1960). Some reports from LA countries are in agreement with these findings. In this continent, G6PD A- is the predominant variant observed among hundreds of healthy male blood donors (Saad et al. 1997b, Compri et al. 2000, Hamel et al. 2002, Oliveira et al. 2009). Moreover, this variant was not a risk factor for moderate hyperbilirubinaemia (Mezzacappa et al. 2010) or anaemia (Cardoso et al. 2012) in Brazil. On the other hand, G6PD A- type deficiency was associated with significant haemoglobin drop in patients using antimalarial preparations containing dapsone in Africa (Pamba et al. 2012).

Nonetheless, these findings do not conclusively indicate that non-specific administration of PQ is safe. To be acceptable, mass drug administration (MDA) needs to heavily rely on the safety of the intervention, as it will reach a majority of healthy individuals (Hsiang et al. 2013, Kondrashin et al. 2014). The use of an unsafe drug for MDA purposes could impose serious hazards to the population, as there is no efficient or accurate rapid diagnostic test currently available. The relatively low number of reported cases of haemolysis from malaria endemic countries that prescribe PQ irrespective of G6PD status assessment may be due to under-diagnosis and under-reporting of this complication, rendering the need for a more thorough assessment of the clinical burden of this condition in the region (Douglas et al. 2012). Should MDA be conducted with PQ in countries with a high prevalence of underlying G6PD deficiency, a high number of adverse events would be expected to occur at the community level, in accordance with the distribution of G6PD variants present in this community. Albeit sporadic, the number of cases of severe haemolysis, haemoglobinuria, acute kidney injury and associated fatalities may not be significant, but they are not negligible. This is especially true in certain geographical areas in which G6PDd rates are high, particularly in those nations where the Mediterranean variant is prevalent. The G6PD Mediterranean variant prevails in certain ethnic groups originating in the Mediterranean and West Asia. Individuals with the G6PD Mediterranean variant have minimal G6PD activity (< 1%) and are predisposed to favism and severe life threatening haemolysis following PQ therapy (Beutler 1991, Howes et al. 2013). In Panama, a mutation analysis revealed that a Mediterranean variant was behind the development of kernicterus after exposure to naphthalene in a male patient (de Gurrola et al. 2008). In Manaus, Brazil, G6PDd was associated with a considerably higher risk of malaria-related transfusions, suggesting that G6PDd may contribute to a considerable proportion of malaria-related complications in an area with an unexpectedly high prevalence of the Mediterranean variant (Ramos-Júnior et al. 2010, Lacerda et al. 2012a, Santana et al. 2013).

A recent systematic review analysed 47 cases of confirmed PQ-induced haemolysis in LA and the Caribbean (Monteiro et al. 2014). Unfortunately, none of these cases had genotyped for G6PD variants.

G6PDd among Amerindian populations - This systematic review analysed 24 publications regarding G6PDd prevalence among Amerindians belonging to 55 ethnic groups living in 12 countries and 64 different villages. A total of 7,205 individuals were tested for G6PDd (2,482 males, 499 females and 4,224 individuals without gender information). Most of this information was generated from anthropological studies on the genetic variability of indigenous groups using the G6PD phenotype as one of the markers. A great interest in this area has allowed the accumulation of valuable information regarding G6PDd prevalence in this ethnic group.

In LA, the first work concerning G6PDd in areas of *P. vivax* circulation found no G6PDd individuals amongst 228 Peruvian Amerindians (Best 1959). Indeed, studies from different areas of this continent confirmed the virtual absence or a very low frequency of G6PDd in Amerindian populations. In this review, only eight deficient individuals were detected among 7,205 screened Amerindians. In Colombia, one Wanauna individual was found to be deficient (Monsalve et al. 1987) and in Mexico, four Amerindians were diagnosed as G6PDd (Lisker et al. 1966). Authors of the last two studies concluded that this could be explained by the introduction of genes from black neighbouring communities. In the Amazon, G6PDd was detected in one Makiritare Amerindian with approximately 20% of normal G6PD enzyme activity in relation to average G6PD activity found in control individuals

from the same village (Mohrenweiser & Neel 1984). The geographic patterns presented here reinforce these findings, with lower G6PDd frequencies observed in Mexico, Argentina, Bolivia and Peru and this finding may be explained by the fact that large portions of the populations in these countries have an Amerindian background.

In parallel with the virtual absence of G6PDd among Amerindian populations, the literature indicates that these groups of people are not susceptible to haemolysis caused by oxidative stressors such as PQ and other drugs. A recent systematic review has shown that among all published cases of haemolysis triggered by various oxidative agents in LA, none have involved Amerindians (Monteiro et al. 2014). Reinforcing these results, in Nicaragua, the administration of 10-20 mg of PQ base during 14 days to 321 Miskito Amerindians with chronic malaria did not lead to any report of secondary haemolysis (Thaeler Jr et al. 1953). This probable safety upon PQ administration in Amerindian population is of great value to malaria control programmes because malaria remains a major health problem in Amerindian villages across this continent.

Safety of PQ use in LA and the Caribbean - The most feared complication of PQ administration is triggering haemolysis in G6PDd individuals. PQ may also cause clinically non-significant haemolysis in non-G6PDd individuals as well. Other major side effects include methemoglobinaemia (MetHb), hypersensitivity reactions and gastrointestinal disturbances (Hill et al. 2006). Because G6PDd screening prior to PQ administration is not usually routine practice in LA or Caribbean countries, the risk of drug-induced haemolytic episodes is always present. Although PQ is the only available drug presenting good efficacy for the treatment of both *P. vivax* and *P. falciparum* primary infections and relapses of *P. vivax* malaria (Baird 2011), it still presents many uncertainties regarding its mode of function, safety and tolerability (Steinhardt et al. 2011) despite having been extensively used for more than 60 years. Safety issues are most concerning in those populations considered most vulnerable, including pregnant women and very young children with uncertain G6PD status, for which the WHO recommends not using PQ due to scarce evidence of its safety (WHO 2010).

Several reports describe haemolytic events associated with PQ use in G6PDd individuals in LA and the Caribbean, including El-Salvador (Bloch et al. 1970), Cuba (Pérez & Meléndez 1989, Menéndez-Capote et al. 1997), Trinidad and Tobago (Chadee et al. 1996), Puerto Rico (McCurdy et al. 1973) and Brazil (Silva et al. 2004, Ramos-Júnior et al. 2010, Lacerda et al. 2012a). A large portion of these patients required blood replacement therapy, presented long-lasting haemolysis and were unable to complete full treatment (Monteiro et al. 2014).

The clinical impact of G6PDd is also important in the Brazilian Amazon, a region accounting for 99.8% of the registered cases of malaria in Brazil (Oliveira-Ferreira et al. 2010). In this region, G6PDd has been associated with an increased risk for malaria-related transfusions (Santana et al. 2009, 2013) and death triggered by PQ-induced haemolysis (Lacerda et al. 2012a). Of note, the standard PQ regimen in Brazil differs from that recommended by the WHO. In an attempt to increase treatment compliance, the Brazilian Ministry of Health, in the mid-1990s, recommended the administration of 30 mg/day of PQ over only seven days (Marques et al. 2001), rather than maintaining the conventional 15 mg/day, 14-day regimen course proposed by the WHO. The decrease in length of PQ treatment to seven days led to an increase in the drug dosage (Silva et al. 2010). The precise consequences of this schedule change on the risk of haemolysis prevalence triggered by PQ in G6PDd individuals remain to be determined.

Other adverse events, such as MetHb, may also occur following PQ therapy. In G6PDd patients, a significantly higher occurrence of MetHb was observed following oral therapy with PQ when compared to non-G6PDd individuals (Santana et al. 2007, Ferreira et al. 2011). In Nicaragua, however, administration of 10-20 mg of PQ base daily over 14 days to 321 Miskito Indians with chronic malaria did not lead to any case of secondary adverse events (Thaeler Jr et al. 1953). LA literature is contradictory in the presentation of adverse events after treatment with PQ. Despite one Colombian study reporting that two and five-fold higher PQ dosages caused only short-term mild-to-moderate side effects (Carmona-Fonseca et al. 2009), another study from this same country reported severe gastrointestinal distress in a small proportion of non-immune male soldiers (Soto et al. 1999).

Most of the countries presenting cases of PQ-induced haemolysis present a higher prevalence of G6PDd when compared to other countries. Although only a few cases are reported in these studies, the real prevalence of clinical haemolytic syndromes is unknown, most likely due to lack of appropriate surveillance. As most studies aim to study the efficacy of PQ regimens against malaria, safety issues are still systematically underexplored. Gaps still need to be filled regarding the presence of serious adverse events due to PQ therapy in both normal G6PD and deficient populations.

Some perspectives concerning rapid diagnostic tests in LA - Several technical and operational knowledge gaps must be addressed to expand access to G6PDd testing and to ensure that a patient's G6PD status is known before deciding to administer PQ (Domingo et al. 2013). A rapid and accurate diagnosis of G6PDd under field conditions before starting PQ treatment is currently a major challenge (malERA 2011, Domingo et al. 2013). A prerequisite to introducing G6PD testing is the availability of high-quality G6PDd tests with product profiles that are compatible with end-use cases. The diagnostic gold standard technique for detecting G6PDd is based on the measurement of NADPH production by the enzyme by means of spectrophotometry, thus requiring a laboratory environment, sophisticated equipment and experienced personnel. For screening activities in less complex environments, the fluorescent spot test has been considered the most appropriate assay, although it requires ultraviolet lamp, bath and micropipette. Given the characteristics described above, none of the currently available G6PDd tests can be routinely employed in field conditions across most malarial areas.

In this context, in 2012, decision-makers gathered at a workshop held in Bangkok, Thailand and indicated that a rapid test for G6PDd should have characteristics similar to rapid tests used in malaria diagnosis: feasibility and ease of interpretation, stability at high temperature and humidity, ability to test on capillary blood, sensitivity and specificity higher than 95% and 75%, respectively, with a cut-off set at 30-40% of the median normal G6PD activity (Domingo et al. 2013).

Although G6PDd systematic testing is currently not performed in malaria endemic areas, there are two rapid tests to detect G6PDd in field conditions: BinaxNOW® G6PD, which is already being commercialised, and CareStart® G6PD, which was recently developed and is now showing promising results (Tinley et al. 2010, Kim et al. 2011, von Fricken et al. 2014). The scarcity of studies on both tests may limit their use by malaria control and eradication programs because there are issues related to their physical and biochemical conservation in the field. High levels of humidity and temperature impact the tests' accuracies by indirectly impairing the establishment of deficiency cut-off points. Better assessments are required to better determine the real usefulness and costs of these tests in LA and Caribbean countries. BinaxNOW® G6PD must be kept between 15-30ºC for storage and between 18-25ºC during performance and this test requires venous blood for the essay, which may increase operating costs and hamper its use. In the United States of America, this test presented high accuracy with 98% sensitivity and 97% specificity, when using a cut-off of < 4.0 U/gHb, corresponding to 49% of normal G6PD activity in the sample (Tinley et al. 2010). Regarding CareStart® G6PD, a study from Cambodia established sensitivity and specificity of 68% and 100%, respectively, at a cut-off of < 3.6 U/gHb (30% of normal activity), demonstrating that the test is not affected by high storage or execution temperatures when performed with samples collected by venipuncture or finger-stick on peripheral blood puncture (Kim et al. 2011).

A standard approach that could define absolute values of normal G6PD activity may be required to validate G6PDd diagnostic tests (Domingo et al. 2013). Typically, G6PDd has been defined as a percentage of normal G6PD activity. No rapid test for G6PD has been assessed in LA malaria endemic epidemiological conditions. In this review of LA and Caribbean studies, a lack of standardisation was noticed in the attempts both to assess normal G6PD activity as well as to define a cut-off for G6PDd diagnosis. Twenty-five community surveys estimated G6PDd prevalence by quantitative methods but, unfortunately, the majority of the studies used inconsistent definitions of normal G6PD activity though different G6PD cut-off points or degrees of G6PDd. Some studies followed manufacturer's definitions of normal activity and G6PDd. Ambiguity in the method of calculation presents practical difficulties in deploying G6PD tests in routine care in LA and Caribbean countries. This ambiguity in interpreting results is particularly problematic in qualitative tests, which highlights that an accurate evaluation of rapid tests is urgently needed in these countries.

From a public health perspective, uncertainty remains in whether G6PD testing deficiency status needs to be taken into account for PQ-based radical cure in some populations, as reflected in the current WHO guidelines. However, from a patient management perspective, where the individual risk/benefit ratio dictates optimal treatment, knowing the G6PD status of the patient is a prerequisite for prescribing an 8-aminoquinoline-based drug (Domingo et al. 2013). Given the scarcity of rapid test evaluations, it becomes a priority to study the use these tests in the diagnosis of G6PD deficiency in tropical areas where malaria is endemic, especially regarding their accuracy. These data are as essential for economic evaluation as for their incorporation by health services. After extensive studies of accuracy, the cost-effectiveness and budget impact of incorporating G6PDd diagnosis in the standard of care for malaria will need to be assessed to support decision-making steps.

A cost-effectiveness analysis is currently the method most commonly used for economic evaluation in health care and in the field of health technology assessment. Such an approach systematically compares costs and consequences of health interventions for prevention, diagnosis or treatment from among the options that have a common purpose (Haddix et al. 2003). The evidence about the cost-effectiveness of interventions is generally used to instruct decisions regarding the allocation of funds from the public sector, helping to identify interventions that represent the best use of resources. In addition to cost-effectiveness analyses, the evaluation of the budget impact can aid decision making by providing financial forecasts if the technology in question is adopted by national health systems (Ribeiro et al. 2012).

Rapid tests have the potential to change radically the healthcare process for patients presenting G6PDd, potentially also allowing the optimisation of available financial resources because deficiency diagnosis likely decreases other costs such as those related to hospitalisations. It is necessary, however, to compare such costs and benefits to those related to possible relapses from *P. vivax* infections and thus determine the importance of G6PDd detection among individuals infected with *P. vivax* through an effective and economically viable rapid test. It is also important to consider a local research agenda in LA to regionally assess the cost and accuracy of rapid tests in field conditions. If rapid tests prove effective in field conditions, studies of cost-effectiveness and budget impact for health services are recommended to support the incorporation of these tests into control programs aimed at malaria elimination in the Western Hemisphere.

Concluding remarks - Malaria elimination will be possible only with serious regional and international efforts addressing asymptomatic infection and persistent *P. vivax* infection. Currently available drugs that can radically cure *P. vivax* malaria and are able to reduce transmission of malaria parasites are those in the 8-aminoquinoline family, such as PQ. Tafenoquine, which is also an 8-aminoquinoline, is currently being assessed as a single-dose radical cure therapy and may represent a significant advance in *P. vivax* therapy. Un-

fortunately, individuals carrying the G6PDd trait are at risk to develop severe haemolysis if exposed to any of these drugs, implying that radical cure regimens will require broader testing for G6PD deficiency.

In conclusion, (i) low prevalence rates of G6PDd were recorded in Argentina, Bolivia, Mexico, Peru and Uruguay. Studies performed in Curaçao, Ecuador, Jamaica, Saint Lucia, Suriname and Trinidad, as well as some surveys carried out in areas of Brazil, Colombia and Cuba showed high prevalence rates (> 10%) of G6PDd, (ii) G6PD encoded by the *G6PD A-^{202A}* mutation is the most broadly distributed genotype across LA, identified in 81.1% of deficient individuals surveyed in the continent, (iii) G6PDd seems to be virtually absent among Amerindians, suggesting that PQ use is safe in these populations and (iv) rapid and accurate diagnosis of G6PDd under field conditions remains a major challenge in LA. The development of a rapid G6PD diagnostic test could help in reducing unnecessary exposure to drugs capable of inducing haemolysis, such as PQ, because LA faces a scarcity of clinical and epidemiological information concerning this enzyme's activity in the population. The development of newer and inexpensive methods for field-testing of G6PD deficiency should go hand in hand with studies of PQ and tafenoquine effectiveness. Reliable exclusion of patients at risk of severe harm opens the possibility for safer and more effective deployment of high dose regimens. If the elimination of *P. vivax* is to be achieved, a coordinated effort will be necessary to provide evidence across the spectrum of *P. vivax* endemic regions and to deploy a safe anti-relapse therapy regimen in an effective manner.

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