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RESEARCH

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Systematic review and individual patient data meta-analysis on glucose- 6 – phosphate dehydrogenase activities measured by a semi-quantitative handheld biosensor

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Abstract

Background Measurement of glucose-6-phosphate dehydrogenase (G6PD) activity guides hypnozoitocidal treatment of *P vivax* malaria. The G6PD Standard (SDBiosensor, Republic of Korea) here referred to as "Biosensor" is a quantitative point-of-care diagnostic that measures G6PD activity in U/gHb. The manufacturer recommends cutoffs to define G6PD deficient (≤ 4.0U/gHb), intermediate (4.1- ≤ 6.0U/gHb) and normal (> 6.0U/gHb) individuals. The aim of this individual patient data (IPD) meta-analysis was to evaluate these cutoffs (CRD42023406595).

Methods A systematic review identified studies reporting population-level G6PD activity measured by Biosensor, published between January 2017 and May 2023. IPD were collated and standardised. The adjusted male median (AMM) was defined as 100% activity and calculated across all studies (universal AMM) and separately for each setting. The proportion of participants classified as deficient or intermediate were compared using the manufacturer-recommended cutoffs and 30% and 70% of the universal AMM and setting-specific AMM. Associations between G6PD activity and blood sampling method, malaria status, and age were assessed.

Results Eleven studies with 9724 participants from eight countries were included in this analysis. The universal AMM was 7.7U/gHb and the setting-specific AMMs ranged from 6.2U/gHb to 9.9U/gHb. When using the universal AMM, 4.2% of participants were classified as deficient and 11.9% as intermediate or deficient. The corresponding values were 3.9% and 10.8% for setting-specific cutoffs, and 7.2% and 18.3% for manufacturer-recommended definitions for deficients and intermediates respectively. The manufacturer-recommended cutoff for deficient individuals fitted the distribution of G6PD activities better than definitions based on the percentage of AMM. There was no significant association between malaria status or blood sampling method and G6PD activity. Measured G6PD activity decreased in children 1–5 years and plateaued thereafter.

Conclusion The manufacturer's recommended cutoff is conservative but more reliable at categorising G6PD deficient individuals than those based on calculations of 30% activity using the AMM. The observed decrease in G6PD activity in children between 1 and 5 years of age warrants further investigation.

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Keywords Plasmodium vivax, G6PD, Dlucose-6-phopsphate dehydrogenase, Diagnostic, Biosensor;, G6PD Standard

Background

Nearly 3.3 billion people are at risk of a *Plasmodium vivax* infection [1, 2]. In contrast to other malaria species, *P. vivax* forms dormant liver stage hypnozoites that relapse weeks to months after a primary infection causing significant morbidity and mortality [3]. These hypnozoites represent a silent reservoir that complicates the elimination of *P. vivax* [4]. The 8-aminoquinolines, primaquine and tafenoquine, are the only licensed drugs that kill hypnozoites; although both drugs can cause severe haemolysis in patients with low glucose-6-phosphate dehydrogenase enzyme (G6PD) activity [5, 6].

G6PD is found in all eukaryotic cells and is essential in human red blood cells (RBCs) to maintain their redox potential [7, 8]. Enzyme activity is measured in international units (U), normalised by a haemoglobin (Hb) measurement (U/gHb) taken at the same time [9]. Since the phenotypic reference method, spectrophotometry, is poorly standardized, the output in U/gHb is converted to percent activity (%) based on the adjusted male median (AMM), to enable comparison across populations [10]. To calculate the AMM, the median activity of all male individuals from the same population is calculated, all observations with values less than 10% of this median are then excluded and the median activity is recalculated from the remaining subset and defined as 100% activity [11]. More than 230 clinically relevant genetic G6PD variants have been described to date, associated with reduced G6PD activities, collectively called G6PD deficiency [7, 12, 13].

The G6PD gene is located on the X-chromosome. Males have one copy of the gene and are hemizygous deficient or normal. Females have two copies and can be either homozygous deficient, homozygous normal, or heterozygous for the gene. Most hemizygous and homozygous deficient individuals have phenotypic G6PD activities below 30% and G6PD normal individuals have activities above 70–80% [14]. In comparison, heterozygous females have a wide range of G6PD activities from deficient to normal [7]. Accordingly, G6PD activities within a population show a bimodal distribution, with activities of the majority of individuals normally distributed around the 100% activity mark and a second smaller peak representing hemizygous and homozygous deficient individuals (Fig. 1) [15].

The World Health Organization (WHO) recommends testing patients for G6PD deficiency prior to treatment with daily primaquine regimens to identify patients with reduced activity and tailor their treatment accordingly [16]. Patients with G6PD activity < 30% should not receive 7- or 14-day primaquine regimens. Tafenoquine is only recommended for patients with ≥ 70% G6PD activity [17–19]. In 2022 the WHO revised their classification of G6PD variants, recommending that any variant with a median activity < 45% (Class B) by spectrophotometry should be considered clinically significant [13, 14, 20]. Although spectrophotometry is the reference method to measure G6PD activity, it is unsuitable for point of care testing [21]. To date, the only point of care test that can reliably discriminate activities between 30% and 70% is the handheld semi-quantitative Biosensor from SD Biosensor (STANDARD G6PD, ROK; Biosensor) [21–24]. The Biosensor generates G6PD (in U/gHb) and haemoglobin (in g/dL) readings from 10 µL of capillary or venous blood within two minutes. The manufacturer recommends thresholds of 4.0 U/gHb and 6.0 U/gHb to discriminate deficient and intermediate activities respectively, although the methods used to select these are not reported [25]. Alignment of these cutoffs with local AMM-derived 30% and 70% cutoffs vary considerably [22]. This individual patient data pooled analysis aimed to assess whether the manufacturer-recommended cutoffs for the Biosensor are universally applicable.

Methods

Search strategy and selection criteria

A systematic review of Medline, Web of Science, Cochrane Central, Scopus, Clinicaltrials.gov and the WHO International clinical trials registry platform was undertaken to identify studies that measured population-based G6PD activity using the G6PD Standard point of care test (Biosensor) manufactured by SD Biosensor (Republic of Korea) between January 1, 2017, and May 17, 2023, in any language. Search terms used were ((G6PD) OR (G6PD deficiency) OR (Glucose-6-phosphate dehydrogenase) OR (Glucose-6-phosphate dehydrogenase deficiency)) AND ((G6PD Standard) OR (SDBiosensor) OR (SD Biosensor) OR (Standard G6PD) OR tafenoquine). Studies were included if they reported activities of at least 36 randomly selected male individuals older than 1 year from a population expected to have a G6PD distribution consistent with the local population. Four reviewers undertook the systematic review (AS, BL, LRV and RJC) with each article screened by two reviewers and discrepancies resolved by discussion. The protocol was registered with PROSPERO CRD42023406595.

Investigators of studies eligible for inclusion were invited to contribute individual patient data in addition to any data from eligible but unpublished studies. Data

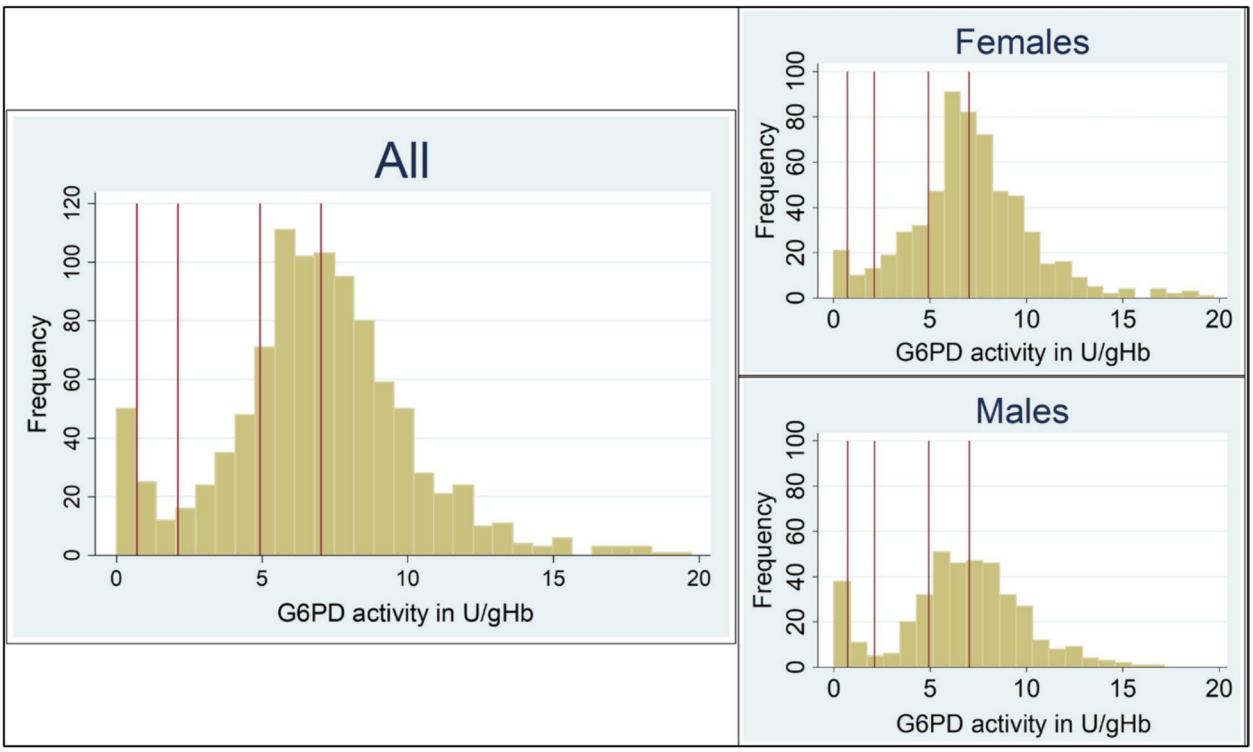


Fig. 1 G6PD activities in samples collected from participants in Bangladesh during a cross-sectional survey measured by spectrophotometry. *Vertical lines from left to right indicate 10%, 30% 70% and 100% G6PD activity* [15]

collected included age, sex, malaria status, G6PD activity (in U/gHb) and haemoglobin (in g/dL) measured by Biosensor, date of G6PD measurement, methodology of blood collection (capillary vs. venous), pregnancy status, date of last malaria episode, and history of giving birth, blood loss or major surgery in the previous 6 months. Individual patient data were excluded if missing age, sex, G6PD activity, or haemoglobin, or if haemoglobin was < 7 g/dL on the Biosensor measurement, as recommended by the manufacturer.

Data were obtained according to ethical approvals from the original study. Our analysis did not require additional ethical approval as the original data approved by ethical review boards were anonymised and unable to be linked to individuals.

Definitions

Each combination of country and study group was defined as a 'setting' and data were analysed per setting. The blood sampling technique was categorised as venous or capillary. The AMM was defined as 100% activity and calculated from male participants above 1 year of age who tested negative for malaria by microscopy or rapid diagnostic test (RDT) and after removal of participants with the lowest 10% of reported G6PD activities [11]. The AMM was calculated for the entire data set (universal AMM) and separately for each setting (setting-specific AMM). G6PD deficient and intermediate status of all included participants were categorised repeatedly: (i) as measured activity of less than 30% and 70% of the universal AMM, (ii) as measured activity of less than 30% and 70% of the setting-specific AMM, and (iii) when applying the manufacturer recommended cut-off of 4.0 U/gHb and 6.0 U/gHb, respectively.

Statistical analysis

All observations were categorised three times based on the different definitions of deficient and intermediate activities and proportions of deficient and intermediate individuals were compared. The distribution of G6PD activities was plotted by sex for the overall population and by setting. The association between blood sampling method (capillary or venous) and G6PD activity was assessed in studies that sampled using both capillary and venous methods. The relationship was analysed using multivariable linear regression adjusting for age, sex, baseline Hb, and malaria status with robust errors and clustering by setting. Using a similar multivariable linear regression model restricted to studies which included participants both with or without malaria,

the association between malaria presence and G6PD activity was investigated. The relationship between age and G6PD activity was also investigated in a similar model. Due to a non-linear relationship between age and G6PD activity, a post hoc analysis was undertaken with a generalised estimating equations model to visualise the relationship between G6PD activity and age with fractional polynomial terms fitted for age, adjustment for age, sex, baseline Hb, and blood sampling method and clustering by setting. This relationship was explored separately in all participants, in males and females. Separate sensitivity analyses were undertaken restricted to males with (i) a haemoglobin > 10 g/dL and (ii) excluding those with an activity ≤ 4.0 U/g Hb (presumed deficient).

The within-study risk of bias assessment was performed using the QUADAS-2 tool [26]. Analyses were undertaken in Stata (version 17).

Results

A total of 322 studies were found in the databases, published between January 1, 2017, and May 17, 2023, of which 300 were excluded after screening titles and abstracts (Fig. 2). A further 18 studies were excluded because G6PD activity was not measured by Biosensor (n = 12), participants were not selected randomly from the whole population (n = 4) or the trial was ongoing (n = 2). A total of four studies met the eligibility criteria [27–30] and data were available and shared by investigators. Data on sex were not recorded consistently across G6PD categories for one study leading to its exclusion. Data from eight studies unpublished as of May 17, 2023, were also available, providing a total of 11 studies on 10,905 participants [25, 31]. Data from 1181 participants were excluded due to missing variables and haemoglobin measurements < 7 g/dL, resulting in 9724 participants from 11 studies included in the analysis.

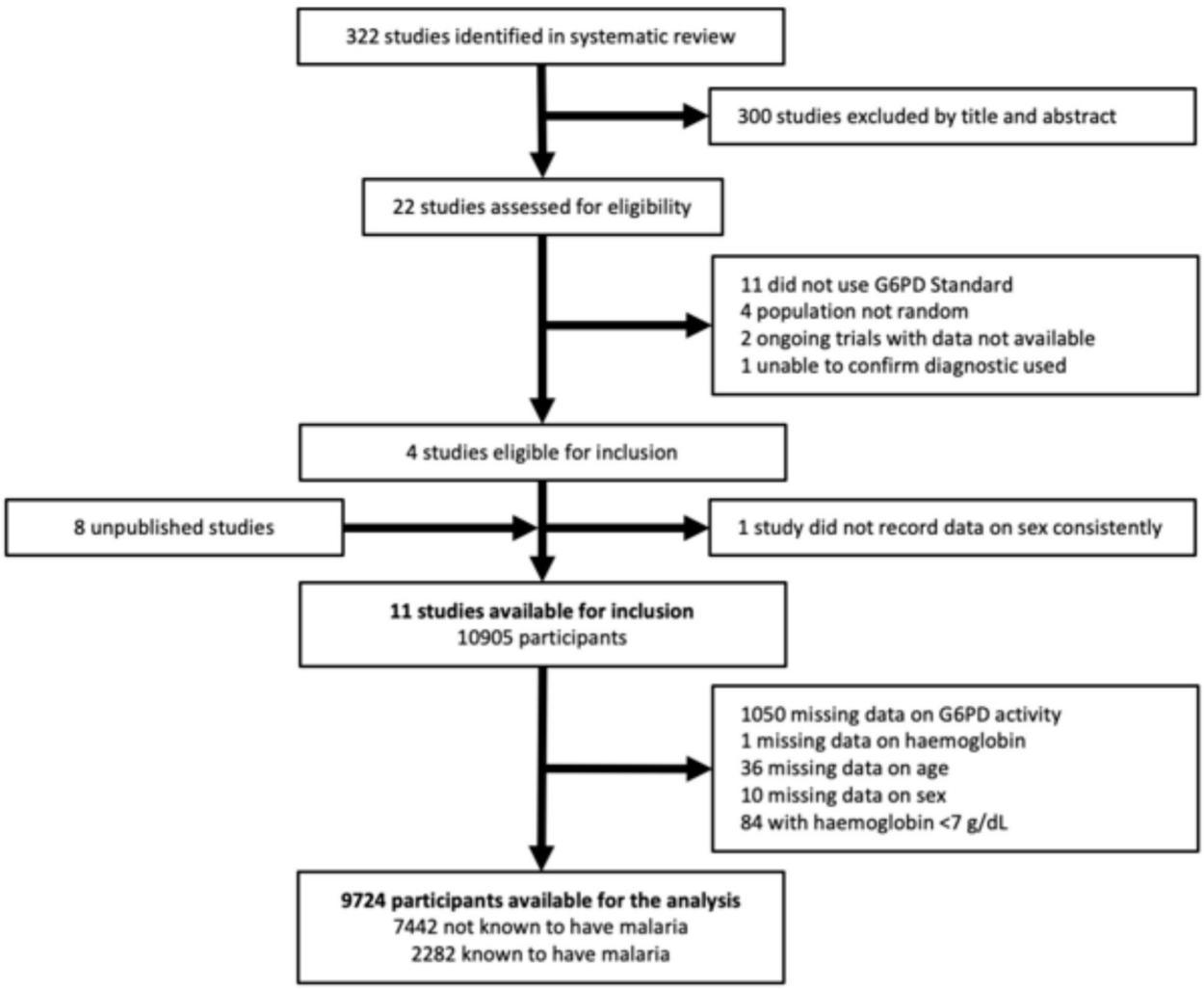


Fig. 2 Flow diagram of patient inclusion

Data were included from 8 countries: Bangladesh, Brazil, Cambodia, Ethiopia, India, Indonesia, Nepal, and the United States of America. By September 22, 2025, only two contributing studies remained unpublished, while 8122 (84%) of the included participants were from published studies. The median age was 30.0 years (interquartile range (IQR) 21.0–43.0), of whom 4237 (43.6%) were female and 8938 (91.9%) were sampled using capillary sampling methods. Malaria was recorded in 2282 (23.5%) participants. No consistent data were available on pregnancy, previous malaria episodes, recent surgery, recent blood loss, or recent blood transfusions. There were 14 unique settings (country and study categories), of which 2 (14%) had unclear or possible risk of bias (Table S1—QUADAS).

The distribution of G6PD activities in male participants showed two peaks consistent with presumed G6PD deficient and G6PD normal populations (Fig. 3). Visually, the nadir in the overlapping region between these peaks occurred at ~ 3.5 U/gHb. The universal AMM, derived from 3959 males without parasitaemia, was 7.7 U/gHb and from this the 30% cutoff was determined to be 2.3 U/gHb and the 70% cutoff was determined to be 5.4 U/gHb (Table 2 and Fig. 3). The AMM calculated for each setting varied substantially from 6.2 U/gHb in one study from Nepal to 9.9 U/gHb in a study in Bangladesh [25, 31]. The setting-specific cutoffs for 30% G6PD activity ranged from 1.9 U/g Hb to 3.0 U/g Hb and the corresponding setting-specific 70% cutoffs varied from 4.3 U/gHb to 6.9 U/gHb (Table 2).

Using the manufacturer's cutoff of 4.0 U/gHb, 7.2% (699) of all participants in this analysis were classified as deficient (72.8% (509) were males), but this decreased to 4.2% (411) when the universal 30% cutoff of 2.3 U/gHb was applied (Fig. 3), and 3.9% (301) when the setting-specific 30% cutoffs were applied (Table 2 and Fig. 4). Using the manufacturer's cutoff of 6.0 U/gHb, 18.3% (1777) of participants were classified as having intermediate or deficient G6PD activity (< 70%) and this decreased to 11.9% (1156) when the universal cutoff was applied and 10.8% (830) when the setting-specific cutoffs were applied. Of the 1078 patients with intermediate activity (> 4.0–6.0 U/gHb) using the manufacturer's cutoffs, 60.1% (648) were male. The distribution of G6PD activities varied substantially between settings (Fig. 4 and Tables 1 and 2).

Two settings sampled more than 10 individuals whose G6PD activity was measured from both capillary and venous samples [25, 31]. After adjusting for age, sex, baseline Hb, and malaria status with clustering by setting, there was no difference in mean G6PD activity between

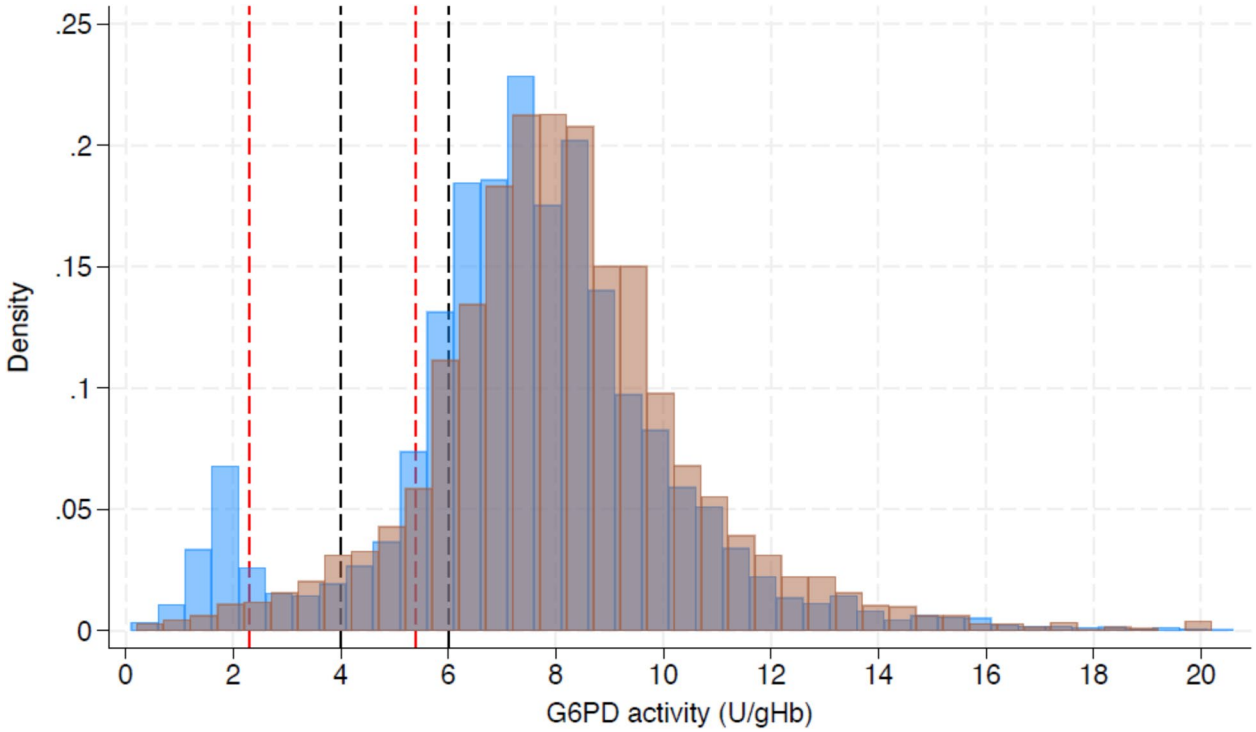


Fig. 3 Density of G6PD activity by sex. Blue bars correspond to males. Orange bars correspond to females. Red dotted lines correspond to 30% and 70% activity of the universal AMM. Black dotted lines correspond to the manufacturer recommended cutoff of 4.0 U/gHb and 6.0 U/gHb

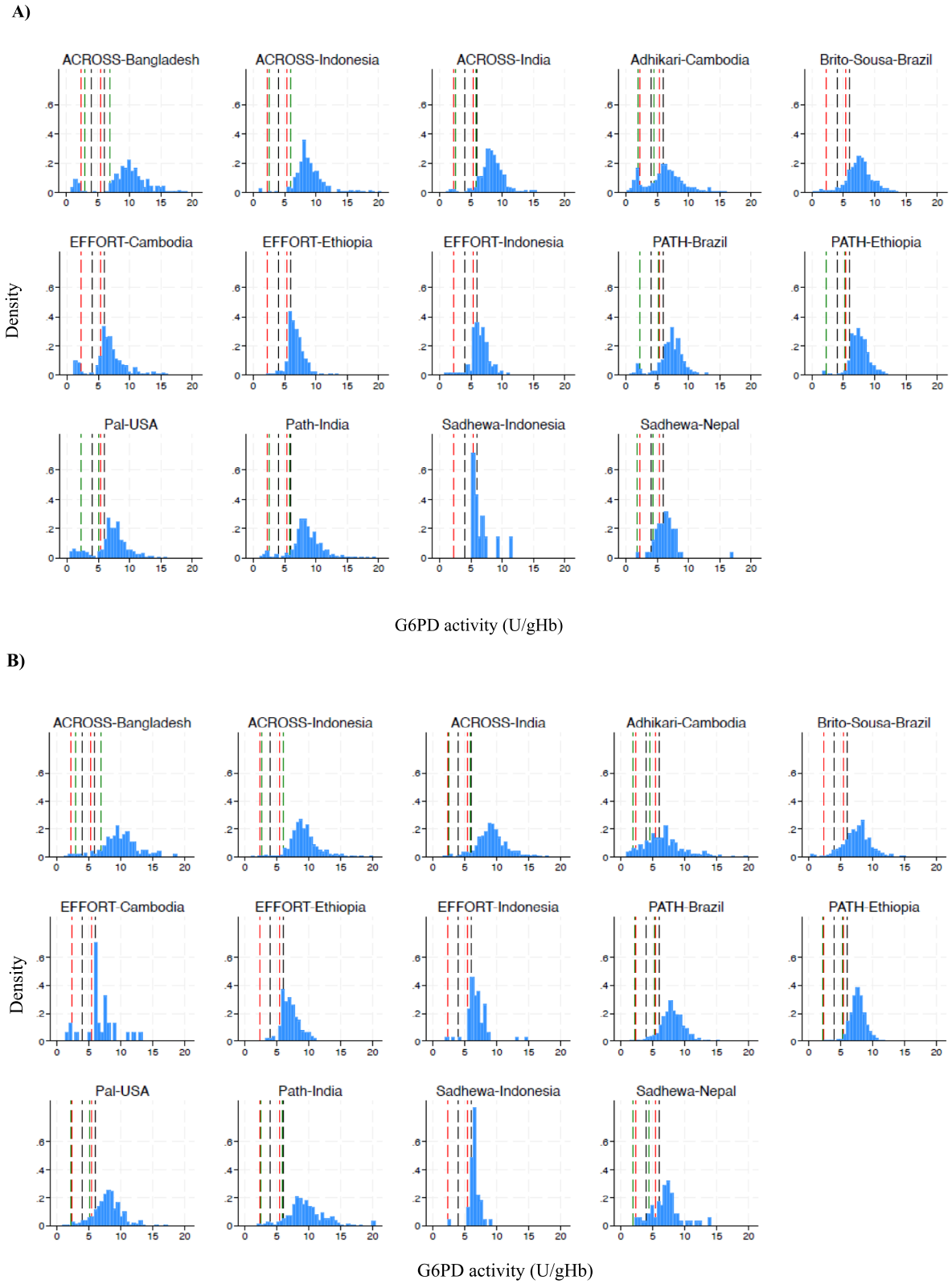


Fig. 4 Density of G6PD activity in **A** males and **B** females by setting. Red dotted lines correspond to 30% and 70% activity of the universal AMM. Black dotted lines correspond to the manufacturer recommended cutoff of 4.0 U/gHb and 6.0 U/gHb. Green dotted lines correspond to 30% and 70% activity of the setting-specific AMM. Setting-specific AMMs were not available for EFFORT in Cambodia, Ethiopia and Indonesia, Sadhewa et al. in Indonesia [31] and Brito-Sousa et al. in Brazil [29]

Table 1 Demographics and baseline measures by setting

Country	Study	Participants	Age	Sex		Malaria present		Blood sampling method		Haemoglobin (g/dL)
				Male	Female	No or unknown	Yes	Venous	Capillary	
Overall		9724	30.0 (21.0–43.0)	5487 (56.4%)	4237 (43.6%)	7442 (76.5%)	2282 (23.5%)	786 (8.1%)	8938 (91.9%)	13.5 (12.0–15.0)
Bangladesh	ACROSS 2019 [25]	468	35.0 (15.0–45.0)	269 (57.5%)	199 (42.5%)	468 (100.0%)	0 (0.0%)	468 (100.0%)	0 (0.0%)	14.4 (13.0–15.8)
Brazil	Brito-Sousa 2022 [29]	1097	23.0 (12.0–39.0)	674 (61.4%)	423 (38.6%)	0 (0.0%)	1097 (100.0%)	0 (0.0%)	1097 (100.0%)	12.9 (11.3–14.4)
	PATH 2019 [25]	1730	37.0 (27.0–47.0)	785 (45.4%)	945 (54.6%)	1480 (85.5%)	250 (14.5%)	8 (0.5%)	1722 (99.5%)	13.2 (12.0–14.5)
Cambodia	Adhikari 2022 [30]	1327	31.0 (23.0–42.0)	993 (74.8%)	334 (25.2%)	1259 (94.9%)	68 (5.1%)	0 (0.0%)	1327 (100.0%)	14.0 (12.4–15.7)
	EFFORT 2023	282	26.0 (21.0–35.0)	251 (89.0%)	31 (11.0%)	5 (1.8%)	277 (98.2%)	0 (0.0%)	282 (100.0%)	14.6 (13.3–15.7)
Ethiopia	EFFORT 2023	373	23.0 (19.0–30.0)	211 (56.6%)	162 (43.4%)	0 (0.0%)	373 (100.0%)	0 (0.0%)	373 (100.0%)	14.0 (12.5–15.5)
	PATH 2019 [25]	1013	24.0 (21.0–31.0)	538 (53.1%)	475 (46.9%)	1013 (100.0%)	0 (0.0%)	0 (0.0%)	1013 (100.0%)	14.6 (13.2–16.2)
Indonesia	ACROSS 2018	752	31.0 (11.0–47.5)	310 (41.2%)	442 (58.8%)	743 (98.8%)	9 (1.2%)	0 (0.0%)	752 (100.0%)	13.5 (12.4–14.9)
	EFFORT 2023	195	28.0 (19.0–40.0)	134 (68.7%)	61 (31.3%)	6 (3.1%)	189 (96.9%)	0 (0.0%)	195 (100.0%)	14.1 (12.6–15.4)
	Sadhewa 2024 [31]	59	35.0 (29.0–42.0)	14 (23.7%)	45 (76.3%)	59 (100.0%)	0 (0.0%)	0 (0.0%)	59 (100.0%)	16.0 (14.6–17.1)
India	ACROSS 2020 [25]	778	33.0 (20.0–47.0)	359 (46.1%)	419 (53.9%)	759 (97.6%)	19 (2.4%)	250 (32.1%)	528 (67.9%)	13.2 (11.7–14.8)
	PATH 2020 [25]	909	35.0 (23.0–50.0)	585 (64.4%)	324 (35.6%)	909 (100.0%)	0 (0.0%)	0 (0.0%)	909 (100.0%)	12.3 (10.8–13.8)
Nepal	Sadhewa 2024 [31]	120	32.0 (21.0–50.0)	51 (42.5%)	69 (57.5%)	120 (100.0%)	0 (0.0%)	60 (50.0%)	60 (50.0%)	14.2 (13.1–16.2)
USA	Pal 2021 [28]	621	34.0 (26.0–48.0)	313 (50.4%)	308 (49.6%)	621 (100.0%)	0 (0.0%)	0 (0.0%)	621 (100.0%)	12.4 (11.4–13.7)

IQR interquartile range

Table 2 Adjusted male medians and cutoffs by setting

Country	Study	Participants	100% AMM, U/gHb	30% AMM, U/gHb	Percentage (n) identified as < 30% cutoff by:			70% AMM, U/gHb	Percentage identified as < 70% cutoff by:		
					Manufacturer (n)	Universal (n)	Setting (n)		Manufacturer (n)	Universal (n)	Setting (n)
Overall		9724	7.7	2.3	7.2% (699)	4.2% (411)	3.9% (301)	5.4	18.3% (1777)	11.9% (1156)	10.8% (830)
Bangladesh	ACROSS 2019 [25]	468	9.9	3.0	7.7% (36)	6.0% (28)	7.1% (33)	6.9	9.6% (45)	8.8% (41)	11.5% (54)
Brazil	Brito-Sousa 2022 [29]	1097	–	–	5.9% (65)	2.6% (29)	–	–	19.1% (209)	11.9% (130)	–
	PATH 2019 [25]	1730	7.5	2.3	5.5% (95)	3.3% (57)	3.1% (53)	5.3	15.5% (268)	9.9% (171)	9.3% (161)
Cambodia	Adhikari 2022 [30]	1327	6.4	1.9	21.5% (285)	13.3% (176)	9.9% (132)	4.5	44.1% (585)	33.8% (449)	24.8% (329)
	EFFORT 2023	282	–	–	11.3% (32)	10.3% (29)	–	–	33.7% (95)	16.7% (47)	–
Ethiopia	EFFORT 2023	373	–	–	2.1% (8)	0.3% (1)	–	–	25.2% (94)	5.1% (19)	–
	PATH 2019 [25]	1013	7.5	2.3	1.9% (19)	0.8% (8)	0.8% (8)	5.3	7.9% (80)	3.7% (37)	3.7% (37)
Indonesia	ACROSS 2018	752	8.6	2.6	2.0% (15)	0.9% (7)	0.9% (7)	6.0	3.9% (29)	2.3% (17)	3.9% (29)
	EFFORT 2023	195	–	–	4.6% (9)	2.1% (4)	–	–	30.8% (60)	9.2% (18)	–
	Sadhewa 2024 [31]	59	–	–	1.7% (1)	1.7% (1)	–	–	28.8% (17)	8.5% (5)	–
India	ACROSS 2020 [25]	778	8.3	2.5	2.3% (18)	1.3% (10)	1.3% (10)	5.8	7.1% (55)	4.9% (38)	6.6% (51)
	PATH 2020 [25]	909	8.3	2.5	5.1% (46)	2.9% (26)	2.9% (26)	5.8	8.6% (78)	6.7% (61)	7.9% (72)
Nepal	Sadhewa 2024 [31]	120	6.2	1.9	7.5% (9)	1.7% (2)	0.8% (1)	4.3	34.2% (41)	25.8% (31)	10.0% (12)
USA	Pal 2021 [28]	621	7.3	2.2	9.8% (61)	5.3% (33)	5.0% (31)	5.1	19.5% (121)	14.8% (92)	13.7% (85)

AMM adjusted male median; An AMM was not calculated for Sadhewa et al. in Indonesia [31] due to too few male participants. An AMM was not calculated for EFFORT in Cambodia, Ethiopia and Indonesia, and Brito-Sousa et al. in Brazil [29] as included participants were predominantly parasitaemic

capillary or venous sampling (mean difference: 0.28 U/gHb, 95% CI − 1.27, 1.83).

Data were available from 6 settings on 5064 participants with and without malaria [25, 31]. After adjusting for confounders, there was no difference in mean G6PD activity between participants with or without malaria (− 0.64 U/gHb, 95% CI − 1.91, 0.64). Similarly, removing parasitaemic participants did not change the percentages of participants classified as having < 30% or < 70% G6PD activity substantially (Table S2).

There was a non-linear association between age and G6PD activity and age and haemoglobin (Fig. S1). In an exploratory analysis, measured G6PD activity decreased sharply with increasing age in children aged 1–5 years and plateaued thereafter, after adjusting for sex, baseline haemoglobin and blood sampling method with clustering by setting (Fig. 5). This relationship was apparent in subgroups of male and female individuals. In sensitivity analyses, the age differences remained apparent when restricted to males with a haemoglobin > 10 g/dL and males with G6PD activity > 4.0 U/g Hb.

Discussion

A reliable and accurate point-of-care diagnostic for G6PD activity is an important tool for the safe prescription of 8-aminoquinoline antimalarial drugs in vivax-endemic countries. This individual patient data pooled meta-analysis of over 9700 participants from 11 studies and eight countries demonstrates substantial heterogeneity in the definition of 100% activity between settings. The current manufacturer-recommended cutoffs to define deficient and intermediate G6PD activity are conservative, placing a higher proportion of individuals in the intermediate or deficient category than when applying locally calculated cutoffs. Definitions for 100% activity based on setting-specific AMMs ranged from 6.2 U/gHb to 9.9 U/gHb between settings and this was reflected by significant variation in the respective 30% and 70% cutoffs. A previous study demonstrated that, under ideal laboratory conditions, with trained laboratory staff, the Biosensor performed comparably across different settings [32]. The differences observed in this analysis could therefore be related to differences in user proficiency or inherent differences between sites.

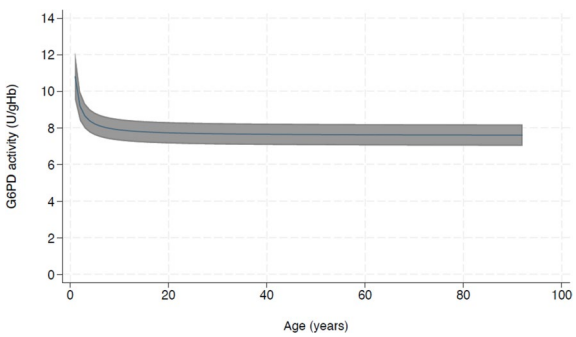


Fig. 5 Relationship between age and G6PD activity in all individuals. Based on a generalized estimating equation model with fractional polynomial terms for age, adjusted for sex, haemoglobin and blood sampling method with clustering by setting after exclusion of participants with haemoglobin < 7 g/dL or > 20 g/dL

The universal definition of 100% activity in the pooled sample was 7.7 U/gHb according to the Biosensor, and the corresponding cutoff for 30% activity (2.3 U/gHb) that was derived from this AMM appeared to exclude a substantial proportion of presumed deficient males from the G6PD deficient group based on the distribution of G6PD activities in the male population (Figs. 3 and 4). In comparison, across all settings most male participants at the lower end of the G6PD activity distributions were included in the deficient group using the manufacturer-recommended cutoff of 4.0 U/gHb (Figs. 3 and 4).

A cutoff at 30% activity defined based on spectrophotometry measurements captures the majority of male deficient patients [14]. In contrast, the Biosensor manufacturer-recommended cutoff of 4.0 U/gHb corresponds to 52% activity as determined by the universal AMM. This difference suggests there is an imperfect correlation between G6PD activity measured by spectrophotometry and G6PD activity measured by Biosensor, such that the correlation is not 1:1. Most qualitative G6PD diagnostics distinguish deficient and normal individuals at the 30% activity level, developed based on results from spectrophotometry, and have effectively guided radical cure in males over several decades [33]. Additional investigation of the relationship between Biosensor and spectrophotometric G6PD activity is warranted including assessment of haemolytic risks at various cutoffs to identify whether adjustments to the manufacturer-recommended cutoff of 4.0 U/gHb to identify G6PD deficient individuals is needed.

The manufacturer recommended cutoff of 6.0 U/gHb corresponds to 78% activity using the universal AMM and thus places a higher proportion of participants in the category of intermediate activity. Treatment with high dose primaquine radical cure (1 mg/kg/day for 7 days) potentially has a greater risk of haemolysis in heterozygous females with intermediate activity [34, 35]. A recent clinical trial in Bangladesh, Ethiopia and Indonesia provided this high dose primaquine radical cure regimen to individuals with ≥ 70% activity based on the local AMM. At one site the corresponding cut-off was 5.0 U/gHb, well below the manufacturer recommended 6.0 U/gHb cutoff for intermediate patients, with no treatment-related serious adverse events observed, and no patients developing severe anaemia (haemoglobin < 5 g/dL) [36]. This study

and previous studies suggest that patients with activities below 6.0 U/gHb may tolerate high dose primaquine and the optimal cutoff to determine heterozygous female patients at risk of severe haemolysis requires further investigation [37]. Our analysis highlights a potential limitation of using setting-specific AMMs to define 100% activity and guide treatment thresholds. In Nepal the AMM was 6.2 U/gHb, which translates into a 70% cutoff of 4.3 U/gHb—substantially lower than the manufacturer's recommended 6.0 U/gHb cutoff. While the median is less affected by outliers, a very high prevalence of G6PD deficiency could result in thresholds that classify individuals with low absolute enzyme activity as eligible for treatment. With increasing availability of the Biosensor, treatment decisions may therefore more appropriately be based on absolute enzyme activity readings (in U/gHb), which might better predict the risk of drug-induced haemolysis. However, in the absence of reliable clinical data on haemolysis it was not possible to correlate Biosensor G6PD activity with haemolytic risks, preventing an understanding of how different cutoffs would perform in a clinical population undergoing 8-aminoquinoline therapy.

Neither the source of blood sampling nor malaria status impacted phenotypic G6PD activity, but G6PD activity did vary with age. Measured G6PD activity decreased from ~ 11 U/gHb to ~ 8 U/gHb as age increased from 1 to 5 years before plateauing thereafter. A decrease in G6PD activity over the first 6–12 months of life had been described previously, however, to our knowledge, this has not been observed in older children [38, 39]. This observation warrants further investigation since it may relate to either a true variation in enzyme activity, other influential parameters such as haemoglobin varying over age, or specific features associated with point of care testing with the Biosensor. If confirmed, this will have important clinical relevance, including the potential for reduced susceptibility to drug-induced haemolysis in young children and hence increased confidence in administering the radical cure in children [40]. If this finding is specific for the Biosensor, it may require an age-based adjustment in results in young children to ensure children are not falsely classified as G6PD normal [41].

This study has several limitations. The proportion of individuals with G6PD deficiency is known to vary substantially between and within countries [42]. Although data from nearly 10,000 individuals from eight countries were included, the study population is not necessarily representative of all populations in vivax endemic regions. Furthermore, setting-specific cutoffs may have been influenced by differences in participant age as well as sample handling, though G6PD activity remains stable for at least 7 days if samples are stored between 4 °C and 8 °C [43, 44]. Data were not available on pregnancy, recent surgery, recent blood loss, or recent blood transfusion, preventing any adjustment for their effect on G6PD activity [7, 45, 46]. The pooled G6PD activity measurements did not have a paired spectrophotometric reference measurement, preventing assessment of systematic bias in specific settings. Due to the absence of a reference method, it was also not possible to compare the performance of universal, and manufacturer recommended cutoffs against a gold-standard method, instead we had to rely on visual inspection of activity distributions.

In the absence of clinical data on haemolysis it was not possible to correlate Biosensor G6PD activity with haemolytic risks, preventing an understanding of how different cutoffs would perform in a clinical population undergoing 8-aminoquinoline therapy.

Conclusion

There was a substantial difference between the manufacturer-recommended cutoffs for G6PD deficient and intermediate individuals and those derived using the current dataset based on 30% and 70% thresholds with the G6PD Standard definitions being more conservative. Further clinical studies are needed to determine the relationship between spectrophotometry and Biosensor G6PD activity measurements and the cutoffs that best predict the risk of drug-induced haemolysis.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12936-025-05654-6.

Additional file 1.

Additional file 2.

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Author contributions

BL developed the concept; BL and RJC developed the methodology; BL, LRV, AS, and RJC collected the data; RJC and MR did the formal analysis; BL wrote the first draft; all authors revised and edited subsequent drafts and approved the final version.

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Data availability

Study data can be obtained by request to the corresponding authors of the included studies.

Declarations

Competing interests
The authors declare no competing interests.

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