Revista Ibero-Americana de Humanidades, Ciências e Educação — REASE

OPEN ACCESS

doi.org/10.51891/rease.v12i8.29619

FLOW CYTOMETRY FOR THE DIAGNOSIS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY: A SYSTEMATIC REVIEW AND META-ANALYSIS

CITOMETRIA DE FLUXO PARA O DIAGNÓSTICO DA DEFICIÊNCIA DE GLICOSE-6-FOSFATO DESIDROGENASE: UMA REVISÃO SISTEMÁTICA E META-ANÁLISE

CITOMETRÍA DE FLUJO PARA EL DIAGNÓSTICO DE LA DEFICIENCIA DE GLUCOSA-6-FOSFATO DESHIDROGENASA: UNA REVISIÓN SISTEMÁTICA Y METAANÁLISIS

Jaime Armando Mandlate [1]
Ruan Matheus Freitas de Castro[2]
Victor Irungu Mwangi[3]
Amanda Carvalho de Oliveira[4]
Marcelo Augusto Mota Brito[5]
Anne Cristine Gomes de Almeida[6]
Yury Oliveira Chaves[7]
Gisely Cardoso de Melo[8]

ABSTRACT: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common hereditary enzymopathy and remains difficult to diagnose, particularly in heterozygous females. Flow cytometry has emerged as a quantitative diagnostic approach capable of detecting deficient erythrocyte populations. This systematic review and meta-analysis evaluated its diagnostic accuracy for G6PD deficiency. Following PRISMA guidelines, PubMed/MEDLINE and the BVS platform, including LILACS, were searched for studies published between 1985 and 2025. Studies comparing flow cytometry with quantitative spectrophotometry and/or genotyping were included. Risk of bias was assessed using QUADAS-2, and pooled sensitivity and specificity were estimated using bivariate random-effects models. Four studies comprising 857 participants (63.1% female) met the inclusion criteria. Flow cytometry demonstrated pooled sensitivity of 92.6% (95% CI: 84.8–96.5) and specificity of 97.2% (95% CI: 90.1–99.3). Although methodological differences contributed to study heterogeneity, the findings consistently showed high diagnostic accuracy. Flow cytometry represents a valuable complement to conventional diagnostic methods, particularly for identifying heterozygous females and supporting safe antimalarial treatment by reducing the risk of drug-induced haemolysis in malaria-endemic settings.

[1] Msc Graduate Student, (Author). Programa de Pós-Graduação em Ciências aplicadas à Hematologia (PPGH-UEA). Universidade do Estado do Amazonas. Manaus, Amazonas, Brazil; Hospital Provincial de Xai-Xai, Gaza, Mozambique.
[2] Msc Graduate Student; (Co-author). Programa de Pós-Graduação em Medicina Tropical (PPGMT-UEA). Universidade do Estado do Amazonas. Manaus, Amazonas, Brazil.
[3] PhD, post doctorate researcher; (Co-author). Programa de Pós-Graduação em Medicina Tropical (PPGMT-UEA). Universidade do Estado do Amazonas. Manaus, Amazonas, Brazil.
[4] PhD Graduate Student; (Co-author). Programa de Pós-Graduação em Medicina Tropical (PPGMT-UEA). Universidade do Estado do Amazonas. Manaus, Amazonas, Brazil.
[5] PhD, Research scientist, (Co-author). Programa de Pós-Graduação em Medicina Tropical (PPGMT-UEA). Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD), Manaus, Amazonas, Brazil.
[6] PhD, Post doctorate researcher, (Co-author). Programa de Pós-Graduação em Ciências aplicadas à Hematologia (PPGH-UEA). Universidade do Estado do Amazonas. Manaus, Amazonas, Brazil; Programa de Pós-Graduação em Medicina Tropical (PPGMT-UEA). Universidade do Estado do Amazonas. Manaus, Amazonas, Brazil.
[7] PhD, Research scientist and professor, (Co-advisor). Programa de Pós-Graduação em Ciências aplicadas à Hematologia (PPGH-UEA). Universidade do Estado do Amazonas. Manaus, Amazonas, Brasil; Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD), Manaus, Amazonas, Brazil; Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas (HEMOAM). Manaus, Amazonas, Brazil; Instituto Leônidas & Maria Deane (ILMD- Fiocruz Amazônia), Manaus, Amazonas, Brazil; Instituto Nacional de Pesquisas da Amazônia (INPA). Manaus, Amazonas, Brazil.
[8] PhD, Graduate program coordinator (PPGMT), Research scientist. (Advisor).Universidade do Estado do Amazonas (UEA); Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD), Manaus, Amazonas, Brazil.

Keywords: Glucose-6-phosphate dehydrogenase deficiency. Flow cytometry. Diagnostic accuracy. Heterozygous females.

RESUMO: A deficiência de glicose-6-fosfato desidrogenase (G6PD) é a enzimopatia hereditária mais comum e permanece de difícil diagnóstico, particularmente em mulheres heterozigotas. A citometria de fluxo surgiu como uma abordagem diagnóstica quantitativa capaz de detectar populações de eritrócitos deficientes. Esta revisão sistemática e meta-análise avaliou a acurácia diagnóstica desse método para a deficiência de G6PD. Seguindo as diretrizes PRISMA, foram realizadas buscas nas bases PubMed/MEDLINE e na plataforma BVS (incluindo LILACS) por estudos publicados entre 1985 e 2025. Foram incluídos estudos que comparavam a citometria de fluxo com a espectrofotometria quantitativa e/ou a genotipagem. O risco de viés foi avaliado utilizando a ferramenta QUADAS-2, e a sensibilidade e especificidade combinadas foram estimadas por meio de modelos bivariados de efeitos aleatórios. Quatro estudos, totalizando 857 participantes (63,1% do sexo feminino), atenderam aos critérios de inclusão. A citometria de fluxo apresentou sensibilidade combinada de 92,6% (IC 95%: 84,8–96,5) e especificidade de 97,2% (IC 95%: 90,1–99,3). Embora diferenças metodológicas tenham contribuído para a heterogeneidade dos estudos, os resultados demonstraram consistentemente alta acurácia diagnóstica. A citometria de fluxo representa um complemento valioso aos métodos diagnósticos convencionais, especialmente para a identificação de mulheres heterozigotas e para viabilizar um tratamento antimalárico seguro, reduzindo o risco de hemólise induzida por medicamentos em áreas endêmicas de malária.

Palavras-chave: Deficiência de glicose-6-fosfato desidrogenase. Citometria de fluxo. Acurácia diagnóstica. Femininas heterozigotas.

RESUMEN: La deficiencia de glucosa-6-fosfato deshidrogenasa (G6PD) es la enzimopatía hereditaria más frecuente y sigue siendo difícil de diagnosticar, especialmente en mujeres heterocigotas. La citometría de flujo ha surgido como un método diagnóstico cuantitativo capaz de detectar poblaciones eritrocitarias deficientes. Esta revisión sistemática y metaanálisis evaluó su precisión diagnóstica para la deficiencia de G6PD. Siguiendo las directrices PRISMA, se realizaron búsquedas en PubMed/MEDLINE y en la plataforma BVS (incluida LILACS) de estudios publicados entre 1985 y 2025. Se incluyeron estudios que comparaban la citometría de flujo con la espectrofotometría cuantitativa y/o el genotipado. El riesgo de sesgo se evaluó mediante la herramienta QUADAS-2, y la sensibilidad y especificidad combinadas se estimaron utilizando modelos bivariantes de efectos aleatorios. Cuatro estudios, que abarcaron a 857 participantes (63,1% mujeres), cumplieron los criterios de inclusión. La citometría de flujo mostró una sensibilidad combinada del 92,6% (IC del 95%: 84,8–96,5) y una especificidad del 97,2% (IC del 95%: 90,1–99,3). Aunque las diferencias metodológicas contribuyeron a la heterogeneidad de los estudios, los resultados demostraron sistemáticamente una elevada precisión diagnóstica. La citometría de flujo constituye un complemento valioso para los métodos diagnósticos convencionales, especialmente para identificar a mujeres heterocigotas y facilitar un tratamiento antipalúdico seguro al reducir el riesgo de hemólisis inducida por fármacos en zonas donde la malaria es endémica.

Palabras clave: Deficiencia de glucosa-6-fosfato deshidrogenasa. Citometría de flujo. Precisión diagnóstica. Mujeres heterocigotas.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common hereditary enzymopathy, affecting over 500 million people worldwide and constitutes a major public health concern in malaria-endemic regions, where accurate diagnosis is crucial for the safe use

of haemolytic antimalarials such as primaquine and tafenoquine (Luzzatto; Ally; Notaro, 2020; Nascimento et al., 2022).

In areas with high disease prevalence, up to 15–20% of males may be G6PD deficient, while a substantial proportion of heterozygous females remain at risk due to the co-circulation of normal and deficient erythrocytes. This underscores the need for effective and reliable screening strategies throughout life, from the neonatal period to adulthood, including during other scenarios of increased exposure to drugs and other oxidative stressors (Bancone et al., 2017). In malaria-endemic regions, accurate determination of a patient's G6PD status across all age groups is essential for the safe use of 8-aminoquinolines and other haemolytic drugs(Zailani et al., 2023).

Currently, quantitative spectrophotometric assays are considered the gold standard for determining G6PD activity. Nevertheless, these methods require specialised laboratory infrastructure and are susceptible to interference from conditions such as anaemia, reticulocytosis, leucocytosis, and recent blood transfusion, which can affect the accuracy of G6PD activity measurements. These limitations are particularly evident in heterozygous females, in whom average enzyme activity may mask the presence of deficient red blood cell subpopulations(Fu et al., 2018; Ley et al., 2017; Peters; Van Noorden, 2009; Sadhewa et al., 2023).

Similarly, qualitative screening tests and some point-of-care assays are limited in their ability to detect intermediate phenotypes, particularly in heterozygous females, thereby perpetuating the risk of drug-induced haemolysis(Fu et al., 2018). Although molecular methods accurately identify G6PD variants, their high cost, limited availability in endemic settings and inability to directly assess enzyme activity restrict their routine application(Garibyan; Avashia, 2013).

Flow cytometry is a promising alternative that enables phenotypic assessment of erythrocytes at the single-cell level, allowing the simultaneous identification of deficient and normal erythrocyte populations within the same blood sample. Studies have shown a strong correlation with spectrophotometric assays and high discriminatory capacity, including the accurate identification of heterozygous females and individuals with mosaic expression patterns(Bancone et al., 2017; Shah et al., 2012).

The cytometric method is based on the conversion of oxyhaemoglobin to its reduced form using reagents such as sodium nitrite, glucose, and the fluorescent dye Nile blue. The subsequent reactions generate a fluorescence signal proportional to intracellular G6PD activity(Kheng et al., 2015; Van Noorden; Dolbeare; Aten, 1989). Erythrocytes with normal

G6PD activity produce sufficient NADPH to suppress Nile blue fluorescence, whereas deficient cells exhibit increased fluorescence intensity, enabling clear discrimination. This principle makes flow cytometry valuable for identifying heterozygous females and individuals with mosaic patterns of enzyme expression, both of whom being difficult to diagnose using conventional enzymatic assays(Shah et al., 2012; Van Noorden; Dolbeare; Aten, 1989).

Several validation studies have reported strong correlations between flow cytometry and spectrophotometric methods, with high diagnostic sensitivity and specificity, particularly in malaria-endemic populations(Satyagraha et al., 2016; Souissi et al., 2025). However, variability in analytical protocols, differences in equipment, fluorochromes, cut-off values, and the reference standards used introduces methodological inconsistencies. These variations hinder direct comparison between studies and limit the establishment of standardised protocols for widespread implementation(Pal et al., 2019; Sadhewa et al., 2023; Souissi et al., 2025). Consequently, there is no clear consensus on the interchangeability of the two methods.

Moreover, no comprehensive synthesis has established robust pooled estimates of the diagnostic accuracy of flow cytometry compared with spectrophotometry and genotyping, particularly for heterozygous females and individuals from malaria-endemic regions(Domingo et al., 2019; Sadhewa et al., 2023). Existing studies report variable estimates of sensitivity, specificity and diagnostic thresholds, highlighting the need for a rigorous evaluation of the available evidence(Domingo et al., 2019). Thus, this study aimed to critically assess the diagnostic accuracy of flow cytometry for detecting G6PD deficiency while determining its clinical utility and relevance as a diagnostic approach.

MATERIAL AND METHODS

Search strategy

A systematic search was conducted in the PubMed/MEDLINE, LILACS and BVS databases using controlled vocabularies from the Medical Subject Headings (MeSH) and Health Science Descriptors (DeCS). The PICO framework was applied and structured into four stages: extraction, conversion, combination, and construction (Supplementary Material Table 1).

The initial search strategy used a combination of the following search terms: ("G6PD" OR "glucose-6-phosphate dehydrogenase deficiency"), "flow cytometry", "diagnosis" AND "sensitivity and specificity". The search terms were adapted to the syntax of each database

while maintaining the underlying Boolean logic. Studies published between January 1985 and August 2025 were considered, with no language restrictions applied. Retrieved records were deduplicated and screened for eligibility.

Inclusion and exclusion criteria

Primary diagnostic accuracy studies conducted in human populations (without restrictions on age or sex) were eligible for inclusion. We included cross-sectional, observational, and diagnostic validation studies evaluating flow cytometry as the index test for detecting glucose-6-phosphate dehydrogenase (G6PD) deficiency. Eligible reference standards comprised quantitative spectrophotometry for G6PD activity, genetic testing (e.g., genotyping or sequencing), or both. To be included, studies had to provide sufficient data to reconstruct a 2 x 2 contingency table for calculating diagnostic accuracy measures.

Studies were excluded if they: (1) did not evaluate flow cytometry as the index diagnostic test; (2) assessed G6PD deficiency using qualitative tests, enzymatic assays without comparison with an appropriate reference standard, or purely genetic profiling lacking phenotypic validation; or (3) were based on *in vitro* or experimental models without human samples. Furthermore, non-original research (e.g., narrative or systematic reviews, editorials, letters to the editor), methodological reports lacking clinical data, and epidemiological studies that failed to report diagnostic accuracy parameters were excluded. Duplicate records and studies with incomplete or unrecoverable data that precluded extraction or reconstruction of data required for the meta-analysis were also removed. Where multi-center or multi-cohort studies provided clearly disaggregated data, each cohort was treated as an independent analytical unit.

Study selection and quality assessment

Screening was carried out by two independent reviewers using the Rayyan platform, version 1.7 (https://new.rayyan.ai/reviews/1580609/overview). Automatic and manual removal of duplicates were performed, followed by screening of titles and abstracts, and a subsequent full-text assessment for eligibility. Discrepancies between reviewers were resolved by consensus. Data on demographic characteristics, methodological features, and diagnostic accuracy outcomes were extracted from the included studies.

The methodological quality of the included studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool (https://mcguinlu.shinyapps.io/robvis/). Four domains were interrogated: patient selection (D1), index test (flow cytometry) (D2), reference standard (D3), and flow and timing (D4). Each domain was assessed for risk of bias. The first three domains were additionally evaluated for applicability concerns, and classified as low risk (+), unclear risk due to missing information (?), or high risk (–). All included studies reported local ethics committee approval. This systematic review was registered in PROSPERO (CRD420251173462).

Statistical analysis

Statistical analyses were performed in R (version 4.5.1). Primary diagnostic accuracy parameters (sensitivity, specificity, false-positive rate, and false-negative rate) were calculated for each study, applying a continuity correction of 0.5 where applicable.

Proportions were transformed using the logit function, and pooled estimates for sensitivity, specificity, and likelihood ratios (LR+ and LR–) with 95% confidence intervals (95% CIs) were derived using bivariate random-effects models. Overall diagnostic performance was evaluated using the area under the receiver operating characteristic curve (AUC) and partial AUC (restricted to observed false positive rates and normalized). Heterogeneity across studies was evaluated using τ^2, bivariate I^2, and Cochran's Q test. Potential publication bias/small-study effects were assessed via funnel plot asymmetry and weighted regression testing. Final model selection was guided by the Akaike information criterion (AIC) and Bayesian information criterion (BIC).

RESULTS

Study selection

Using the pre-defined MeSH terms, 814 articles, published between 1985 and 2025, were identified from the PubMed/MEDLINE and LILACS-BVS databases. Subsequently, 98 duplicates were removed and a further 669 articles excluded for not meeting the study eligibility criteria, mainly not using flow cytometry as a diagnostic method for G6PD.

Consequently, a total of 47 articles underwent full-text assessment. Of these, 30 were excluded due to lack of diagnostic accuracy data, use of inappropriate reference standards, or inability to reconstruct 2×2 contingency tables. The remaining 17 articles were then evaluated

for methodology, of which 6 were excluded (4 did not meet the G6PD-specific eligibility criteria, and 2 did not use spectrophotometry as a comparison method). From the remaining 11 studies, 7 were excluded due to methodological limitations, incomplete data, inappropriate study design, or populations that did not meet the review criteria (Figure 1). Overall, four studies fulfilled all eligibility criteria and were included in the quantitative synthesis and meta-analysis and were subjected to risk-of-bias assessment and applicability concerns using the QUADAS-2 tool (Figure 2).

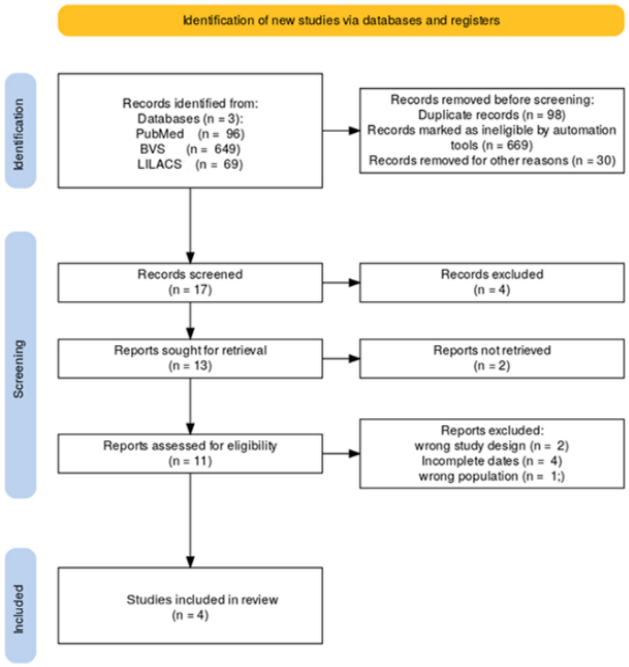


Figure 1: PRISMA flow diagram showing the database search, screening process and selection of studies included in the systematic review and meta-analysis.

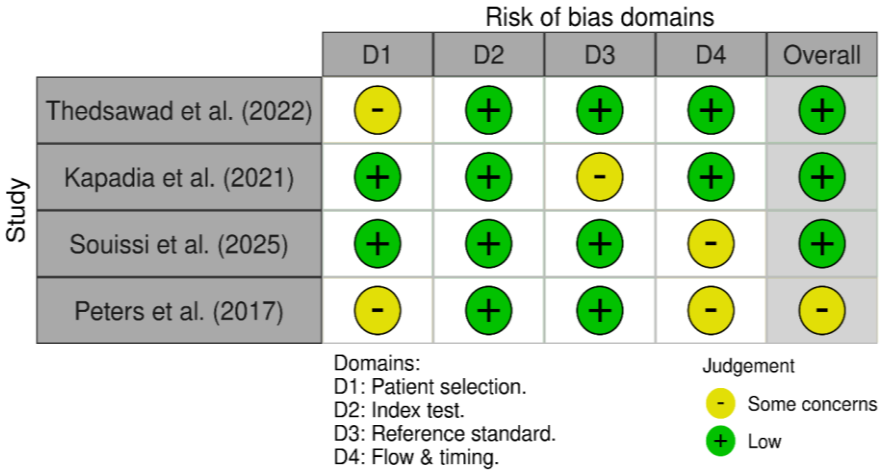


Figure 2: Risk-of-bias assessment in the selected studies using QUADAS-2. Tool: https://mcguinlu.shinyapps.io/robvis/.

GENERAL CHARACTERISTICS OF THE INCLUDED STUDIES

The four studies, comprising 946 individuals, covered different geographical locations (India, Thailand, France, and the Netherlands). Participants' ages ranged between 0 and 65 years, and the majority (63.1%; 597/946) were females. Of the 946 individuals, only 857 (90.6%) underwent flow cytometric evaluation to determine their G6PD status.

Of the 857 individuals evaluated via flow cytometry, 507 (59.2%) were women, of whom 334 (39.0%) exhibited normal G6PD activity, 75 (8.8%) were classified as deficient, 83 (9.7%) showed intermediate activity, 9 (1.0%) were classified as atypical, and 6 (0.7%) had inconclusive results. Among the 345 (40.3%) men evaluated, 255 (29.7%) had normal G6PD activity, 87 (10.2%) were classified as deficient, and 8 (0.4%) classified as atypical (**Table 1**).

Table 1: Characteristics of the included studies and distribution of G6PD status by sex and country.

					Males			Females					
Author	**Year**	**Country**	**Age**	**Total (M/F)**	**Normal G6PD (%)**	**Deficient G6PD (%)**	**Atypical (%)**	**Normal G6PD (%)**	**Deficient G6PD (%)**	**Intermediate G6PD (%)**	**Atypical (%)**	**Inconcl. (%)**	**Number evaluated by FC (%)**
Thedsawad et al.	2022	Thailand	>18 years	205 (80/125)	55 (69.0)	25 (31.3)	-	81 (65.0)	4 (3.2)	37 (30.0)	-	-	202 (98.5)
Kapadia et al.	2021	India	0-65 years	62 (20/42)	0	20 (100.0	-	20 (47.6)	0	22 (52.4)	-	-	62 (100.0)
Souissi et al.	2025	France	>18 years	514 (249/265)	200 (80.3)	42 (16.9	8 (3.2)	185 (69.8)	42 (15.8)	24 (9.0)	9 (3.4)	-	510 (99.2)
Peters et al.	2017	Netherlands	> 3 months	165 (F)	-	-	-	48 (29.0)	29 (18)	-	-	6 (3.6)	83 (50.3)
Total (%)				**946**	**255 (29.7)**	**87 (10.2)**	**8 (0.4)**	**334 (39.0)**	**75 (8.8)**	**83 (9.7)**	**9 (1.0)**	**6 (0.7)**	**857 (90.6)**

Note: Total (M/F) - the absolute number of participants among male (M) and females (F); Males=349, Female=597; FC - flow cytometry; Inconcl. – Inconclusive

Among cases with atypical profiles, fluorescence distributions displayed multimodal patterns, indicating the presence of mixed erythrocyte populations. The exact percentage of G6PD-positive red blood cells (%RBC G6PD+) was not specified in the study. Only one case

was confirmed as heterozygous for G6PD deficiency in a woman carrying the A- variant; in the remaining atypical cases, molecular confirmation was not performed, and the results were therefore considered inconclusive(Peters et al., 2017; Souissi et al., 2025).

Flow cytometric evaluation was performed using different platforms and instruments, which included the BD LSR Fortessa, the BD FACSCanto II with three lasers, the Navios EX (Beckman Coulter), and the BD LSR II. This instrumental variability demonstrates the methodological heterogeneity among the included studies (**Supplementary Material Table 2**). Classification of G6PD status was based on comparison of the flow cytometry results with reference methods, predominantly quantitative spectrophotometric assessment of enzymatic activity, using study-specific cut-off values. Overall, these studies focused on method validation by comparing flow cytometric assays with comparators such as the methaemoglobin reduction test and confirmatory enzymatic assays of G6PD activity (**Supplementary Material Table 3**).

BETWEEN-STUDY HETEROGENEITY

Substantial heterogeneity was observed across the studies for both sensitivity (I^2 = 97.2%, p<0.001) and specificity (I^2 = 99.3%, p<0.001). Although flow cytometry maintained strong pooled performance, this high variance suggests that operational factors across settings significantly impact diagnostic accuracy.

ANALYSIS FOR PUBLICATION BIAS

Funnel plot asymmetry testing via a mixed-effects meta-regression model revealed no significant evidence of small-study effects or publication bias for either pooled specificity (p=0.54) or sensitivity (p=0.88) (**Figure 3**).

PRIMARY ACCURACY MEASURES OF CYTOMETRY

Across the included studies, diagnostic accuracy was consistently high, with sensitivity ranging from 83.9% to 97.8% and specificity from 86.7% to 98.8% (**Table 2**). Variances in point estimates reflect methodological and population differences across studies.

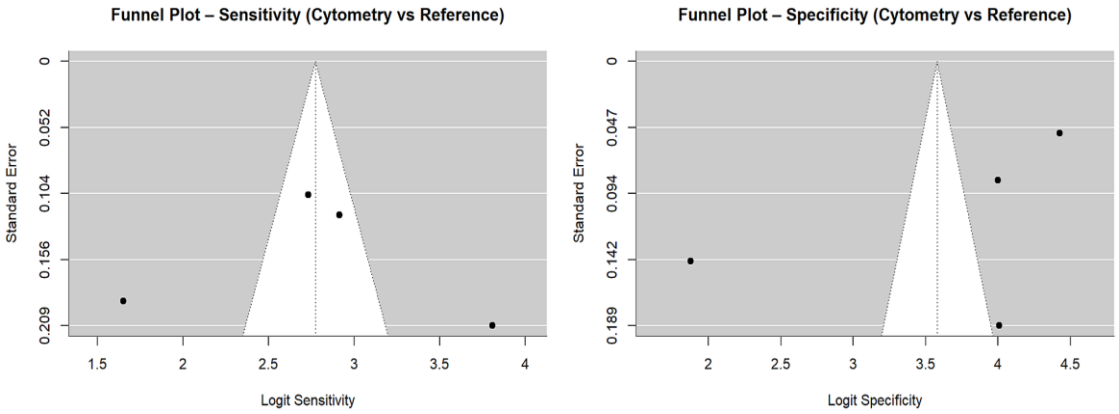


Figure 3: Funnel plot and regression test for assessing asymmetry in the diagnostic accuracy meta-analysis of flow cytometry. Each point represents an individual study included in the meta-analysis, based on its sensitivity and specificity estimates.

Table 2: Primary accuracy measures of flow cytometry, adjusted with a continuity correction of 0.5.

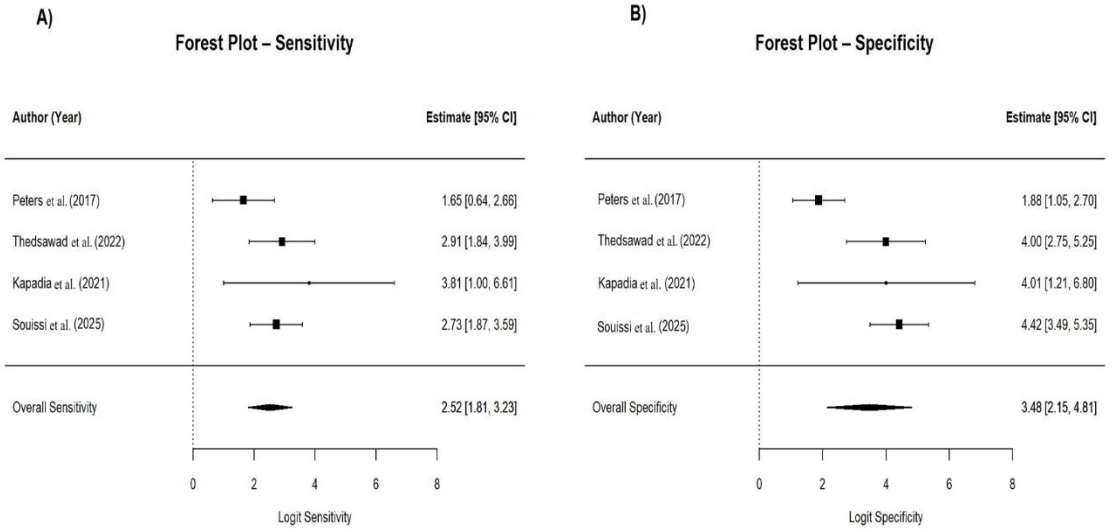
Author	TP	FP	FN	TN	Sensitivity	Specificity
Thedsawad et al. (2022)	64.5	2.5	3.5	136.5	0.949	0.982
Kapadia et al. (2021)	22.5	0.5	0.5	27.5	0.978	0.982
Souissi et al. (2025)	84.5	4.5	5.5	375.5	0.939	0.988
Peters et al. (2017)	23.5	6.5	4.5	42.5	0.839	0.867

Note: TP = true positives; FP = false positives; FN = false negatives; TN = true negatives.

POOLED DIAGNOSTIC ACCURACY ANALYSIS

Across the four evaluated studies, flow cytometry demonstrated high overall diagnostic accuracy for identifying G6PD deficiency compared to reference assays. The pooled sensitivity was 92.6% (95% CI: 86–96%) (**Figure 4a**), and the pooled specificity was 97.0% (95% CI: 90–99%) (**Figure 4b**), reflecting a low false-positive rate. Flow cytometry robustly distinguished G6PD-deficient from normal individuals, supported by a pooled AUC of 0.971, and with a partial AUC of 0.937 which is still very high when limited to the observed data. The assay exhibited a superior diagnostic capacity, a diagnostic odds ratio of 468.2, underscoring the assay's robust capacity to discriminate between G6PD-deficient and G6PD-normal individuals with minimal misclassification.

Figure 4: Forest plot of individual and pooled estimates for a) sensitivity and b) specificity in the selected studies



evaluating flow cytometry for the detection of G6PD deficiency. Estimates are presented on the logit scale with corresponding 95% confidence intervals. Squares represent the point estimates for each study, and the diamond represents the pooled logit value from the meta-analysis.

DISCUSSION

Significant variation was observed between studies regarding the cut-off values adopted for classifying enzyme deficiency and normality. Previous studies comparing flow cytometry with spectrophotometry, which is considered the reference method for G6PD activity assessment, have reported strong correlations, with Pearson correlation coefficients ranging from 0.857 to 0.918 (Ley et al., 2017, 2019). These findings support the validity of flow cytometry as a reliable quantitative method for G6PD phenotyping.

The clinical utility of flow cytometry is particularly evident in the identification of heterozygous females. In a Thai cohort, flow cytometry classified individuals into three phenotypic groups based on the proportion of bright cells: normal homozygotes (>85.4% bright cells), heterozygotes (6.3–85.3%), and deficient homozygotes (<6.2%). When compared with genotyping, the assay showed a sensitivity of 93.2% and a specificity of 100% (Thedsawad et al., 2022).

In contrast, spectrophotometric assays performed on whole blood haemolysates provide only the average enzymatic activity of the erythrocyte population and may fail to accurately identify heterozygous individuals. Approximately 30% of heterozygous females exhibit enzyme activities exceeding 70% of the adjusted normal mean and may therefore be misclassified as G6PD normal (Chu et al., 2017). This limitation is clinically relevant because the haemolytic risk in heterozygous women is influenced by the proportion of G6PD-deficient

erythrocytes resulting from X-chromosome lyonisation. Individuals with a substantial fraction of deficient cells, particularly those with residual activity below 30%, remain susceptible to oxidative drug-induced haemolysis despite having apparently normal overall enzyme activity (Watson et al., 2018).

A recent meta-analysis that evaluated quantitative G6PD assays for the detection of heterozygous females reported a pooled area under the receiver operating characteristic curve (AUC) of 0.86 (95% CI: 0.81–0.90), with an optimal threshold of 60% of normal enzyme activity, corresponding to a sensitivity of 74% and a specificity of 88% (Sociedade Brasileira de Pediatria, 2022). However, the performance of these assays is influenced by methodological variability and by factors such as reticulocyte counts, limiting their generalisability across different populations and laboratory settings. In comparison, the pooled analysis showed superior diagnostic accuracy, highlighting the high discriminatory capacity of flow cytometry for distinguishing G6PD-deficient individuals from G6PD-normal individuals and reinforce its value as a robust diagnostic approach, particularly in populations where accurate identification of heterozygous females is clinically important.

Patient selection was a primary area of concern, given the wide variation in study sample sizes (n = 1–514) (LaRue et al., 2014; Souissi et al., 2025). Additionally, participants were selected on the basis of clinical suspicion or family history, which may introduce selection bias and limit the generalisability of the results (Peters et al., 2017). There were also concerns regarding the reference standards, whereby one study reported discrepancies in the classification of heterozygous women, underscoring limitations in the standardisation and reliability of the reference methods (Kapadia et al., 2021). Flow and timing also raised concerns. The method used in the study by Souissi et al. (2025) was optimised for analysis up to 28 days after sample collection. However, the requirement for immediate analysis within 30 minutes after hydrogen peroxide addition may be challenging in high-throughput laboratories(Souissi et al., 2025). Additionally, although the study was conducted over 18 months, there were delays in the collection of additional samples for certain tests (such as chromate inhibition). In the study by Peters et al. (2017), the need to repeat flow cytometry because of inconclusive results may prolong the total diagnostic turnaround time (Peters et al., 2017).

The standard reference methods used in the selected studies varied. These included the haemoglobin reduction method with a cut-off value for G6PD deficiency defined at ≤ 10%. When enzymatic activity assays were used as the reference standard, Kapadia et al. (2021)

defined normal activity as ≥ 5.03 U/g Hb, intermediate activity as 2.12–3.31 U/g Hb, and G6PD deficiency as ≤ 1.38 U/g Hb (Kapadia et al., 2021). However, after applying local reference controls to adjust the diagnostic thresholds, they established a cut-off value of 1.66 U/g Hb (Kapadia et al., 2021; Thedsawad et al., 2022).

Methodological differences in the definition of normal G6PD activity ranges may complicate direct comparisons across studies. For instance, a paediatric study applied age-specific reference intervals, defining normal activity as 9.2–13.3 U/g Hb in children over two years of age and 9.1–27.1 U/g Hb in those under two years of age (Souissi et al., 2025). Despite such variability in reference standards, these differences did not appear to affect the overall diagnostic performance of flow cytometry, particularly with respect to sensitivity and specificity.

These findings are consistent with evidence from studies conducted in Thailand and the United States, where flow cytometry showed diagnostic performance comparable to that of reference assays for detecting G6PD deficiency, defined as <30% of normal activity. In these settings, the method achieved 100% sensitivity (95% CI: 95.7–100) and 97% specificity (95% CI: 94.5–98.5). Moreover, it showed strong discriminatory capacity for identifying women with intermediate deficiency (<70% activity), with a sensitivity of 95.5% (95% CI: 89.7–98.5) and a specificity of 97% (95% CI: 94.5–98.6). Together, these data support the robustness of flow cytometry as a reliable diagnostic approach across diverse populations and methodological frameworks (Pal et al., 2019).

Compared with a meta-analysis that evaluated the performance of qualitative point-of-care tests in a rural malaria-endemic area of Indonesia using a cut-off of 30% of normal G6PD activity (Satyagraha et al., 2016), the pooled specificity observed for flow cytometry showed a slightly lower specificity. In the Indonesian study, the G6PD RDT showed a sensitivity of 100% and a specificity of 98.7% in men, compared with 91.7% and 92.4% for the standard qualitative fluorescent spot test (FST). In women, the G6PD RDT showed a sensitivity and a specificity of 83.3% and 92.7% respectively, compared with a sensitivity of 100% and a specificity of 92.2% for the FST at the 30% threshold (Satyagraha et al., 2016).

Despite the limited number of studies included in this review (four studies), the findings should be interpreted with caution, as meta-analyses based on few studies are more susceptible to publication bias, particularly due to the exclusion of studies with negative or neutral results, which may lead to overestimation of effect estimates(Pereira; Galvão, 2014). Our analysis found no evidence of significant small-study effects or publication bias affecting

the pooled sensitivity or specificity estimates across studies, despite the use of different flow cytometer platforms. Similarly, a meta-analysis including 11 studies, all evaluating the performance of point-of-care quantitative tests for measuring G6PD activity with spectrophotometry as the reference standard, also found no evidence of publication bias based on pooled specificity and sensitivity estimates (Sadhewa et al., 2025). Similar findings were reported in another review that included eight studies, which also found no evidence of substantial publication bias(Ley et al., 2019). It is worth noting that some studies were classified as having a high risk of bias, mainly due to intentional participant selection and inadequate temperature control during spectrophotometry (Henriques et al., 2018). Collectively, these findings suggest that the limited number of included studies did not substantially affect the pooled estimates of the diagnostic performance of flow cytometry for detecting G6PD deficiency in venous blood samples.

Random-effects meta-analyses revealed substantial heterogeneity across studies for both sensitivity and specificity. For both measures, the between-study variance was more likely to reflect true differences among studies rather than sampling error. Despite the high heterogeneity in diagnostic performance across studies, the pooled estimates indicate that the flow cytometry retains overall diagnostic accuracy, although its performance may vary across different settings or populations. Therefore, the observed variability between studies cannot not be attributed solely to chance (Higgins; Thompson, 2002).

This variation between studies is not unique to flow cytometry. Similar between-study variability has also been reported in a meta-analysis evaluating the quantification of G6PD activity via spectrophotometry using commercial kits from Pointe Scientific, Randox and Sigma-Aldrich. Significant inter-laboratory variations in assay precision and in replicate measurements could be likely due to variability in laboratory methods and undetermined population factors (Pfeffer et al., 2020).

In the present meta-analysis, the observed between-study heterogeneity likely reflects differences in the methodology and study populations. These include variation in testing sites, flow cytometer models, sample preparation protocols, and the type and quality of reference standards used. Ethnic and genetic differences between populations may also contribute to this variation.

Such factors may also explain the lower specificity reported in some studies, which could be influenced by differences in instrumentation, population characteristics, and the cut-off thresholds applied. In addition, heterogeneity may reflect underlying genetic and

phenotypic diversity across geographically distinct populations, as well as the inclusion of women with suspected heterozygous G6PD deficiency in some studies, a group known to exhibit more variable enzyme activity profiles (Peters et al., 2017).

Although the evaluated studies were generally consistent, the confidence intervals around the pooled estimates suggest that these findings may have been influenced by the limited sample sizes (Kapadia et al., 2021). Despite the between-study variation in instrumentation, population characteristics, and the cut-off thresholds applied, the pooled estimates support the overall diagnostic accuracy and potential applicability of flow cytometry for the diagnosis of G6PD deficiency (Oliveira et al., 2010). Laboratories with established infrastructure and experience in flow cytometry tend to exhibit lower result variability, whereas laboratories with less standardized procedures may show greater inconsistency. Differences between cytometer models and settings, such as laser and filter configurations, may influence G6PD activity readings in individual erythrocytes. Notably, with continuous training, the use of appropriate controls, and robust quality control protocols, consistent diagnostic performance can be achieved.

CONCLUSIONS

With over 90% sensitivity and specificity, alongside significant between-study heterogeneity, flow cytometry shows high overall diagnostic performance for detecting G6PD deficiency. These results support the potential use of flow cytometry as a diagnostic tool, particularly for the rapid identification of G6PD deficiency among heterozygous women, in whom conventional methods may have limited sensitivity. Finally, the substantial heterogeneity observed across studies limits the generalisability of the reported diagnostic estimates and protocols to different flow cytometry settings. Thus, implementation of the method should be supported by local validation and regular external quality control procedures.

ACKNOWLEDGMENTS

We wish to thank all participants included in the studies we examined, as well as all researchers and staff who collected the primary data that enabled this review. We also thank Samuel Ndegwa (KEMRI) for his assistance in contextualising the analysis. We also acknowledge the PDJ scholarship (175855/2023-4) by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and PIPD-1/2024 scholarship by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) awarded to VIM.

FUNDING

No financial support or any other form of monetary compensation, direct or indirect, was received related to the conduct of this systematic review and meta-analysis.

DATA AVAILABILITY

Primary accuracy data were extracted from the studies for statistical calculations and analysis. The data used in the paper is available in the Supplementary Material.

ETHICAL APPROVAL

None required.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTION

JAM: Conception and design of the study, Methodology, Data collection, Formal analysis and data interpretation, Writing – original draft, Writing – review & editing; **RMF:** Conception and design of the study, Methodology, Data collection, Formal analysis and data interpretation, and Writing – review & editing; **VIM:** Methodology, Data collection, Formal analysis and data interpretation, and Writing – review & editing; **ACO:** Data collection, Data interpretation, and Writing – review & editing; **MAMB:** Conception and design of the study, Data interpretation, and Writing – review & editing; **ACGA:** Conception and design of the study, Methodology, Data interpretation, Supervision, Writing – review & editing; **YOC:** Conception and design of the study, Methodology, Data interpretation, Supervision, Writing – review & editing; **GCM:** Conception and design of the study, Methodology, Data interpretation, Supervision, Writing – review & editing.

REFERRENCES

BANCONE, Germana *et al.* The G6PD flow-cytometric assay is a reliable tool for diagnosis of G6PD deficiency in women and anaemic subjects. Scientific Reports, v. 7, n. 1, 2017.

CHU, Cindy S. *et al.* Haemolysis in G6PD Heterozygous Females Treated with Primaquine for Plasmodium vivax Malaria: A Nested Cohort in a Trial of Radical Curative Regimens. PLoS Medicine, v. 14, n. 2, p. e1002224, 2017.

DOMINGO, Gonzalo J. *et al.* Addressing the gender-knowledge gap in glucose-6-phosphate dehydrogenase deficiency: Challenges and opportunities. International Health, v. 11, n. 1, p. 7-14, 2019.

FU, Chunyun *et al.* Newborn screening of glucose-6-phosphate dehydrogenase deficiency in Guangxi, China: Determination of optimal cutoff value to identify heterozygous female neonates. Scientific Reports, v. 8, n. 1, p. 833, 2018.

GARIBYAN, Lilit; AVASHIA, Nidhi. Polymerase chain reaction. Journal of Investigative Dermatology, v. 133, n. 3, p. 1–4, 2013.

HENRIQUES, Gisela *et al.* Comparison of glucose-6 phosphate dehydrogenase status by fluorescent spot test and rapid diagnostic test in Lao PDR and Cambodia. Malaria Journal, v. 17, n. 1, p. 243, 2018.

HIGGINS, Julian P. T.; THOMPSON, Simon G. Quantifying heterogeneity in a meta-analysis. Statistics in Medicine, v. 21, n. 11, p. 1539–58, 2002.

KAPADIA, Alpeshkumar Bipinbhai *et al.* Evaluation of a flow cytometric test for G6PD-deficient erythrocytes. Trop Med Int Health, v. 26, n. 4, p. 462–468, 2021.

KHENG, Sim *et al.* Tolerability and safety of weekly primaquine against relapse of Plasmodium vivax in Cambodians with glucose-6-phosphate dehydrogenase deficiency. BMC Medicine, v. 13, n. 1, p. 203, 2015.

LARUE, Nicole *et al.* Comparison of quantitative and qualitative tests for glucose-6-phosphate dehydrogenase deficiency. American Journal of Tropical Medicine and Hygiene, v. 91, n. 4, p. 854–61, 2014.

LEY, Benedikt *et al.* Methods for the field evaluation of quantitative G6PD diagnostics: A review. Malaria Journal, v. 16, n. 1, p. 311, 2017.

LEY, Benedikt *et al.* Performance of the Access Bio/CareStart rapid diagnostic test for the detection of glucose-6-phosphate dehydrogenase deficiency: A systematic review and meta-analysis. PLoS Medicine, v. 16, n. 12, p. e1002992, 2019.

LUZZATTO, Lucio; ALLY, Mwashungi; NOTARO, Rosario. Glucose-6-phosphate dehydrogenase deficiency. Blood, v. 136, n. 11, p. 1225–1240, 2020.

NASCIMENTO, Joabi Rocha *et al.* Prevalence of glucose 6-phosphate dehydrogenase deficiency in highly malaria-endemic municipalities in the Brazilian Amazon: A region-wide screening study. Lancet Reg Health Am, v. 12, p. 100273, 2022.

OLIVEIRA, Glória Maria de *et al.* Revisão sistemática da acurácia dos testes diagnósticos: uma revisão narrativa. Revista do Colégio Brasileiro de Cirurgiões, v. 37, n. 2, p. 153–156, 2010.

PAL, Sampa *et al.* Evaluation of a novel quantitative test for glucose-6-phosphate dehydrogenase deficiency: Bringing quantitative testing for glucose-6-phosphate dehydrogenase deficiency closer to the patient. American Journal of Tropical Medicine and Hygiene, v. 100, n. 1, p. 213–221, 2019.

PEREIRA, Mauricio Gomes; GALVÃO, Taís Freire. Heterogeneidade e viés de publicação em revisões sistemáticas. Epidemiologia e Serviços de Saúde, v. 23, n. 4, p. 775–778, 2014.

PETERS, Anna L. *et al.* Comparison of Spectrophotometry, Chromate Inhibition, and Cytofluorometry Versus Gene Sequencing for Detection of Heterozygously Glucose-6-Phosphate Dehydrogenase-Deficient Females. Journal of Histochemistry and Cytochemistry, v. 65, n. 11, p. 627–636, 2017.

PETERS, Anna L.; VAN NOORDEN, Cornelis J. F. Glucose-6-phosphate Dehydrogenase Deficiency and Malaria: Cytochemical Detection of Heterozygous G6PD Deficiency in Women. Journal of Histochemistry & Cytochemistry, v. 57, n. 11, p. 1003–1011, 2009.

PFEFFER, Daniel A. *et al.* Quantification of glucose-6-phosphate dehydrogenase activity by spectrophotometry: A systematic review and meta-analysis. PLoS Medicine, v. 17, n. 5, p. e1003084, 2020.

SADHEWA, Arkasha *et al.* A Review of the Current Status of G6PD Deficiency Testing to Guide Radical Cure Treatment for Vivax Malaria. Pathogens, v. 12, n. 5, p. 650, 2023.

SADHEWA, Arkasha *et al.* Performance of quantitative point-of-care tests to measure G6PD activity: An individual participant data meta-analysis. PLoS Neglected Tropical Diseases, v. 19, n. 3, 2025.

SATYAGRAHA, Ari W. *et al.* Assessment of Point-of-Care Diagnostics for G6PD Deficiency in Malaria Endemic Rural Eastern Indonesia. PLoS Neglected Tropical Diseases, v. 10, n. 2, p. e0004457, 2016.

SHAH, Shivang S. *et al.* A novel cytofluorometric assay for the detection and quantification of glucose-6-phosphate dehydrogenase deficiency. Scientific Reports, v. 2, n. 299, 2012.

SOCIEDADE BRASILEIRA DE PEDIATRIA, Departamento Científico de Hematologia e Hemoterapia (2019-2021). Deficiência de Glicose -6- Fosfato Desidrogenase – G6PD. *[S.l.: S.n.]*. Available in: https://www.sbp.com.br/deficiencia-de-glicose-6-fosfato-desidrogenase-g6pd/. Acessed on: 21 jul. 2026.

SOUISSI, M. *et al.* Glucose-6-phosphate dehydrogenase deficiency detection using fluorocytometric assay: Evaluation after 1 year of clinical implementation. Cytometry Part B - Clinical Cytometry, v. 108, n. 2, p. 161–171, 2025.

THEDSAWAD, Anchalee *et al.* Cut-off values for diagnosis of G6PD deficiency by flow cytometry in Thai population. Annals of Hematology, v. 101, n. 10, p. 2149–2157, 2022.

VAN NOORDEN, C. J. F.; DOLBEARE, F.; ATEN, J. Flow cytofluorometric analysis of enzyme reactions based on quenching of fluorescence by the final reaction product: detection of glucose-6-phosphate dehydrogenase deficiency in human erythrocytes. The journal of histochemistry and cytochemistry: official journal of the Histochemistry Society, v. 37, n. 9, p. 1313–1318, 1989.

WATSON, James *et al.* Implications of current therapeutic restrictions for primaquine and tafenoquine in the radical cure of vivax malaria. PLoS Neglected Tropical Diseases, v. 12, n. 4, p. e0006440, 2018.

ZAILANI, Mohamed Afiq Hidayat *et al.* Population screening for glucose-6-phosphate dehydrogenase deficiency using quantitative point-of-care tests: a systematic review. Frontiers in Genetics, v. 14, p. 1098828, 2023.

SUPPLEMETARY FILES

Supplementary Table 1: Process of constructing search descriptors.

Objective	To evaluate the diagnostic accuracy of flow cytometry for the detection of G6PD deficiency, compared with spectrophotometric methods (reference standard) and genotyping.			
	P (population)	**I (intervention)**	**C (context)**	**O (outcome)**
Extraction	Participants subjected to the G6PD test	Flow Cytometry	Evaluation of the G6PD enzyme using flow cytometry.	Diagnostic accuracy (sensitivity and specificity).
Convertion	G6PD, Glucose-6-phosphate dehydrogenase deficiency	Flow Cytometry	Diagnostic evaluation	Sensitivity, Specificity
Combination	G6PD OR glucose-6-phosphate dehydrogenase deficiency	Flow Cytometry	Diagnosis	Sensitivity AND Specificity
Construction	"G6PD" OR "glucose 6 phosphate dehydrogenase deficiency") AND ("citometria de fluxo" OR "flow cytometry" AND "diagnóstico" OR "diagnosis" AND "sensibilidade AND especificidade" OR "sensitivity AND specificity"			

Supplementary Table 2: Methodological characteristics of the included studies.

Author	**No. of Participants**	**Equipment used**	**Reference Method**	**Methodological characteristics**

Thedsawad et al. (2022)	205 (M=80; F=125)	BD LSR-Fortessa (BD Biosciences)	Quantitative methaemoglobin reduction test (MRT). Genomic DNA was extracted from a CPDA-preserved blood sample using the Gentra Puregene Blood Kit (QIAGEN, Hilden, Germany). Multiplex ARMS-PCR was performed for genotyping, followed by sequencing. Haematological parameters were measured using a Sysmex XN-3000 automatic haematology analyser (Sysmex Corporation, Kobe, Hyōgo, Japan).	Flow cytometry using the Nile Blue dye in washed red blood cells, compared with the quantitative methaemoglobin reduction test (MRT) and genotyping (multiplex ARMS-PCR followed by sequencing). Cut-off values were defined by the percentage of brightly fluorescent cells, and diagnostic accuracy was assessed in terms of sensitivity, specificity, and area under the receiver operating characteristic curve (AUC).
Kapadia et al. (2021)	62 (M=80; F=125)	BD FACS Canto II (BD Biosciences)	Methaemoglobin reduction test (MRT) using the Enzopak MT kit (Reckon Diagnostic, Vadodara, India) for the assessment of G6PD enzyme activity; confirmatory G6PD enzyme activity assay (EAA)	Flow cytometry based on ferryl-haemoglobin generation following the reduction of methaemoglobin with potassium cyanide (KCN) and hydrogen peroxide (H_2O_2), compared with the methaemoglobin reduction test (MRT) and the quantitative G6PD enzyme activity assay; erythrocytes were classified by the percentage of brightly fluorescent cells, and correlation and agreement between the methods were assessed.
Souissi et al. (2025)	514 (M=80; F=125)	Navios EX (Beckman Coulter, USA)	Spectrophotometric assay using a Specord 205 spectrophotometer (Analytik Jena, Germany).	Prospective cohort assessing flow cytometry using Nile Blue on the Navios EX platform, compared with spectrophotometry and, in selected cases, molecular analysis of the *G6PD* gene; cut-off values were defined by the percentage of G6PD-positive red blood cells (%RBC G6PD+), and diagnostic performance was evaluated using confusion-matrix metrics, receiver operating characteristic (ROC) curve area under the curve (AUC), and methodological quality by QUADAS-2.

Peters et al. (2017)	165 (F=125)	LSRII (BD Biosciences)	Spectrophotometry: based on the Zürcher method. Chromate inhibition assay. Genetic sequencing: a custom AmpliSeq panel (Thermo Fisher Scientific) was used.	Prospective study comparing spectrophotometry (Zürcher method with chromate inhibition), flow cytometry according to the Shah (2012) protocol (using Nile Blue staining and methaemoglobin reduction with KCN and H_2O_2), and *G6PD* gene sequencing (AmpliSeq) as the reference standard in women with suspected or heterozygous deficiency; diagnostic sensitivity and specificity were assessed, and risk of bias was evaluated using QUADAS-2.

Note: M- Male; F- Female

Supplementary Table 3: Individual results by included study

Author	Country	% Bright cell	Sens.	Spec.	AUC	Reference standard	Molecular test (variant-G6PD)	Concordance
Thedsawad et al. (2022)	Thailand	Normal ranges: Female: 85.4–100%; Male: 76.5–100%. Female (homozygotes): 0–6.2%; Male (hemizygotes): 0–76.4%	95.5	98.6	**Female:** Normal vs. Heterozygotes: 0.993 Heterozygotes vs. Homozygotes: 0.992 **Male:** Normal vs. Hemizygotes: 1.000	Quantitative MR test: G6PD normal: %MR > 10%. G6PD deficient: %MR ≤ 10%.	Most common: G6PD Viangchan (871G>A): 9.8%. G6PD Canton (1376G>T): 8.3%. G6PD Kaiping (1388G>A): 4.9%. G6PD Mahidol (487G>A): 4.4%.	Flow cytometry versus multiplex ARMS-PCR: kappa coefficient = 0.944. Flow cytometry versus methaemoglobin reduction test (MRT): agreement (kappa) = 1.000.
Kapadia et al. (2021)	India	Males and Female: Normal >85%; Deficient < 10%	100	100	No information	MRT Normal: ≥5.03 U/g Hb. Intermediate: 2.12–3.31 U/g Hb. Deficient: ≤1.38 U/g Hb. Enzymatic assay activity EAA: cut-off :1.66 U/g Hb,	Genotyping not done	Flow cytometry showed 90% agreement with the methaemoglobin reduction test (56/62 cases) and 77% agreement with the enzyme activity assay (48/62 cases)
Soussi et al. (2025)	France	Normal > 98%, intermediate 3%-99.9%, and	94.4	98.9	0.978	Normal: 9.2–13.3 U/g Hb (under 2 years) and 9.1–27.1 U/g Hb (over 2 years).	Variants A- (most common). Other rare variants: Union/Maewo/	High agreement between methods, k=0.94 (95% CI: 0.90–0.98) for the

		deficient < 5% (Male and Female)				G6PD/PK ratio <0.5 was considered G6PD deficiency	Chinese 2 and Seattle.	entire population.
Peters et al. (2017)	Netherlands	Normal: 10.15% (mean+3SD) Deficient: <10.15%	85	88	No information	**Normal:** 4.80 IU/g Hb (mean). **Deficient:** 2.23 IU/g Hb (mean). **Intermediate/ heterozygous:** 4.39 IU/g Hb (mean).	G6PD Aures (Class 2, 48 Ile → Thr) G6PD A- (Class 3, 68 Val → Met; 126 Asn → Asp) G6PD Chatham (Class 2, 335 Ala → Thr) G6PD Gaohe (Class 2, 32 His → Arg) G6PD Modena (Class 3, 282 Asp → His) G6PD Nefza (Class 3, 323 Leu → Pro) G6PD Sassari/Cagliari (Class 2, 188 Ser → Phe)	Agreement between methods: **Sensitivity**: • Spectrophotometry: 0.52 (95% CI: 0.32–0.71). • Chromate inhibition: 0.96 (95% CI: 0.71–1.00). • Flow cytometry: 0.85 (95% CI: 0.66–0.96). **Specificity:** • Spectrophotometry: 1.00 (95% CI: 0.93–1.00). • Chromate inhibition: 0.98 (95% CI: 0.89–1.00). • Flow cytometry: 0.88 (95% CI: 0.75–0.95). Chromate inhibition showed the highest sensitivity and specificity among the methods tested.

Note: Sens- Sensitivity; Spec – Specificity; AUC – Area under the curve; MRT – Methaemoglobin reduction test; %MR – Percentage of methaemoglobin reduction